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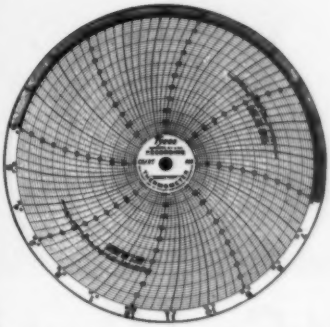

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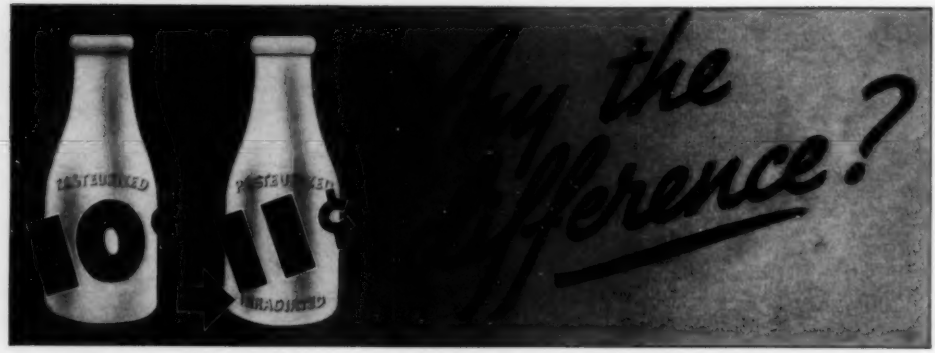

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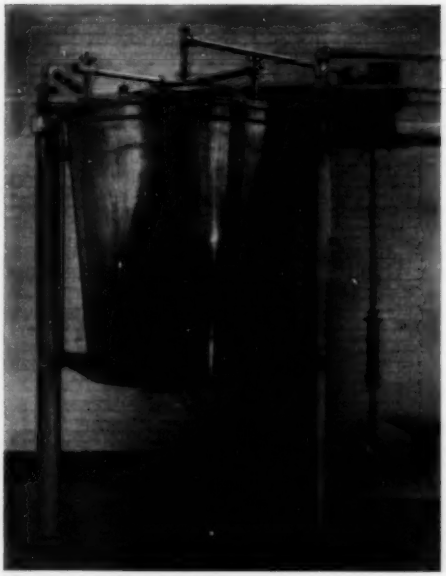

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JOURNAL OF DAIRY SCIENCE

VOLUME XIX MARCH, 1936 NUMBER 3

NORMAL VARIATIONS IN THE CURD TENSION OF MILK[1]

W. H. RIDDELL, W. J. CAULFIELD AND C. H. WHITNAH
Kansas Agricultural Experiment Station, Manhattan

In the course of an investigation (3) started in the summer of 1932 to study some of the factors influencing the Hill curd test, opportunity was given to observe some of the normal variations in the curd character of milk. The milk of each cow in the Kansas Agricultural Experiment Station dairy herd was tested for curd tension at monthly intervals over an extended period. It soon was evident that more variation in curd tension prevailed than the available literature would indicate. This raised the question as to how frequently tests should be run on a group of cows to insure the production of milk of strictly soft curd character. Since conclusive data were lacking on the influence of a number of other factors, the present investigation was outlined to study (1) daily variation in the curd tension of milk from individual cows, (2) influence of stage of lactation, (3) influence of breed, (4) variations in milk composition and curd tension following freshening, and (5) correlation between curd tension and the protein content of milk.

Alleman and Schmid (1), in an early study, found the individuality of the cow to be an important factor influencing curd character. Most of our information on the variations in the curd character of milk, however, have come from the pioneer observations of Hill (9). More recently Berry (2) and Doan and Welch (5) have reported on the influence of such factors as the individual milking, colostral period, season and stage of lactation.

EXPERIMENTAL

The technique followed was that recommended by Hill (8), with the one modification suggested by Monier and Sommer (10) of adding the 10 c.c. of coagulant to the coagulation cylinder before the milk is introduced. This method was found to be fully as accurate and more rapid than the original Hill procedure. A water bath, accommodating 36 coagulation cylinders, facilitated the running of a large number of samples under carefully controlled conditions. Caulfield and Riddell (3) have shown temperature and

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[1] Contribution No. 101, Department of Dairy Husbandry, and No. 193, Department of Chemistry.

time of cutting to be important factors influencing the accuracy of the test. All tests were run in triplicate at 35° C. and the more recently developed curd-o-meter,[2] which cuts down through the curd, was used in cutting all samples.

DAILY VARIATIONS IN CURD TENSION

Data are presented in Table I on the daily variation in curd tension over a 10-day period for representative cows of soft, medium and hard curd tendencies. These results are typical of the fluctuations in curd tension over a limited period of time. While Hill has designated milk of 20 grams curd tension or less as soft curd, the number of cows testing this low in any herd is so extremely limited that 30 grams or less has been accepted as the standard for soft curd milk in a majority of cases. Furthermore, there is a current impression that a single test is sufficient to identify the curd character of a cow's milk and that this value holds throughout the lactation and the cow's lifetime. The first three cows listed in Table 1 were producing milk of approximately soft curd character. An average coefficient of variation of approximately 20 per cent is indicated for these cows. The daily variation in curd tension is of sufficient magnitude to warrant rather careful selection of the producing unit. It would seem desirable, therefore, to make several consecutive tests on a group of cows before final selection, if milk of soft curd character is to be produced.

TABLE 1

Daily variation in curd tension values within periods of 10 days

COW NO.	AVERAGE VALUE	COEFFICIENT OF VARIATION	RANGE
	(*grams*)		(*grams*)
1	19.1	23.5	12– 27
2	28.0	15.1	22– 34
3	32.4	17.1	20– 42
4	40.0	13.8	32– 48
5	65.0	17.7	44– 84
6	83.5	7.5	74– 94
7	91.4	9.8	78–104

INFLUENCE OF STAGE OF LACTATION ON CURD TENSION

The data on the influence of stage of lactation on curd tension are presented in Table 2. Complete lactations were not available on all cows due to differences in time of freshening. However, all lactations involved were at least five months in length and the figures in each case represent the average of the data available for each month. These results can be taken as rep-

[2] Heusser Instrument Manufacturing Co., The American Curd-O-Meter. Bulletin 54. 1932.

resentative since the average figures obtained for 21 complete lactations of 10 months or longer show the same general trend.

It will be observed that there is a tendency for the curd tension to increase from the second month to the end of the lactation. A general scrutiny of the data shows that the curd tension does not remain uniform throughout the lactation, although there is no pronounced increase in successive months and the curd tension values may be fairly uniform for a period of several months. This condition applies also in the case of typically soft curd producers. It will be noted that from the second through the fourth month, the last group in Table 2 would hold this classification. Throughout the remainder of the lactation, particularly from the eighth month to the end of the period, a more rapid increase took place, with the result that the average curd tension was more than 100 per cent higher in the tenth month than in the second.

These findings are not in agreement with those of Hill (9) and Berry (2), both of whom reported that excluding the beginning and final months the curd tension remains fairly constant throughout the lactation period. Furthermore, these results would emphasize the necessity of making at least two or three tests of the milk throughout the lactation of each cow in the soft curd group if the uniformity of the product is to be guaranteed in this respect.

Occasionally samples of milk which failed to set a curd were obtained from cows well advanced in lactation. This fact also has been commented upon by Hill (9).

TABLE 2

Influence of stage of lactation on curd tension (in grams)

NO. LACTATIONS AVERAGED	MONTH OF LACTATION											
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th
86 partial	79	40	46	51	56	56	59	62	62	64	66	75
21 complete	77	45	54	55	65	60	62	67	70	73	70	79
18 (soft curd)	58	24	27	34	41	40	40	47	54	58	53	67

CHANGES IN CURD TENSION VALUES AND MILK COMPOSITION FOLLOWING PARTURITION

Hill (9) reported that milk is considerably higher in curd tension during the first month or six weeks than later. Berry (2) found in colostrum studies on 26 cows that the milk reaches a normal curd tension by the tenth day. Since the number of cows producing soft curd milk is quite limited, it is important to know how soon after freshening the milk of such cows is available for consumption.

The data in Table 3, giving individual results on 8 cows, show the fluctuations in curd tension values following freshening. These figures

indicate that while the milk of some individuals has attained uniform curd tension values at the end of 10 days, in a majority of cases at least two to three weeks were necessary for this to take place. Attention is directed to the irregularity in behavior of curd tension values for the first few days following parturition. In a few instances the highest curd tension is registered with the initial colostrum, followed by an irregular decrease until uniformity is attained. In the majority of cases, maximum values were observed on the third to fifth days following freshening. This irregular behavior in curd tension following parturition probably can be related to the major variations in the serum solids portion of the secretion, particularly in the protein and ash constituents.

In a parallel study of the changes in composition of milk following parturition, some interesting results were secured, which can be used, in part, to explain the changes in curd tension. These data are presented in Table 4 and, so far as the authors are aware, are the most extensive observations on the changes in the major constituents of milk for breeds in this country for approximately the first month after freshening. While some attention has been paid to this phase of lactation by other workers, the data are limited. Crowther and Raistrick (4) reported analyses of the proteins of milk for the first eight milkings after freshening. Engel and Schlag (6) made rather comprehensive determinations on the milk of one cow, showing the changes of colostrum into milk over a period of 7 days. The figures in Table IV represent averages of determinations on 7 cows over the first 28 days of their lactation.

The first milk is an especially rich secretion averaging approximately twice as rich in total solids content as the later normal milk. This, undoubtedly, depends to a considerable extent on the length of the dry period and the level and nature of the feeding during this period. The striking feature about the colostrum is the extremely high globulin content. Crowther and Raistrick (4) have reported the presence of about 0.03 per cent globulin in normal milk. It will be noted that as late as the 28th day after freshening the value for globulin is still appreciably higher than normal. Average values for albumin, casein and ash are all considerably higher in the first milk, while the sugar is approximately 50 per cent of normal. The fat, on the other hand, shows no consistent change. In general the most rapid decline in all constituents, with the exception of the sugar, has taken place within the first four milkings following parturition. The milk sugar has made the most rapid increase within this same period. It will be observed from the data in Table III that the curd tension reaches a maximum average value at approximately the same time. Espe and Dye (7) and Weisberg, Johnson and McCollum (11) have demonstrated a close correlation between the amount of casein in milk and the resulting curd tension. The influence of the mineral portion and the concentration and manner of dispersion of

TABLE 3

Changes in curd tension values following parturition (in grams)

SAMPLE TAKEN	I. (HOL.)	II. (HOL.)	III. (HOL.)	IV. (HOL.)	V. (AYR-SHIRE)	VI. (AYR-SHIRE)	VII. (JERSEY)	VIII. (JERSEY)	AV. CURD TENSION
1st milking	117	20	63	115	82	40	154	30	77
2nd "	94	46	28	144	84	36	133	34	75
3rd "	95	112	21	100	96	51	153	32	83
4th "	67	118	48	83	95	84	178	44	90
3rd day	81	100	45	103	96	114	139	93	97
4th "	73	104	38	92	96	123	112	76	89
5th "	58	89	28	71	126	94	88	84	80
7th "	56	69	28	78	52	54	73	70	60
10th "	38	48	26	55	36	54	91	72	53
14th "	36	40	10	55	39	39	78	53	44
21st "	30	32	19	32	36	42	73	51	39
28th "	30	36	21	40	29	40	83	52	41

the fat on the curd character also has been pointed out. In the present study a correlation of $0.76 \pm .04$ was obtained between the curd tension and the total protein content of 58 samples of normal milk from the Kansas Agricultural Experiment Station herd of the four major breeds. This would indicate that the protein is a major factor producing differences in curd tension, but that other factors also have a modifying influence.

In view of the high correlation between the casein and curd tension in normal milk, the question may be raised as to why the curd tension values do not correlate more closely with the casein in the initial 3 or 4 milkings. (See Tables III and IV). From the fourth milking through the remainder of the first month's lactation the correlation between the casein or total protein and curd tension is more regular. In the early milkings, the high globulin and albumin content of the milk undoubtedly are modifying influences. As their concentration decreases at a very much more rapid rate than does the casein, the latter soon becomes the dominant factor influencing curd tension.

TABLE 4

Changes in composition of milk following parturition[1]

(Values in per cent)

SAMPLE TAKEN	ALBUMIN	GLOBULIN	CASEIN	ASH	SUGAR	FAT	T.S.[2]
1st milking	2.62	7.90	6.99	1.26	2.62	5.4	26.79
2nd "	1.66	5.44	6.12	1.10	3.23	4.9	22.45
3rd "	.48	2.19	4.16	.91	4.18	5.0	16.92
4th "	.55	1.27	3.97	.90	4.32	4.1	15.11
3rd day	.28	.72	3.98	.89	4.49	4.5	14.86
4th "	.39	.49	3.62	.84	4.61	4.2	14.15
5th "	.17	.47	3.17	.81	4.72	4.5	13.84
7th "	.22	.40	2.90	.79	4.67	4.4	13.38
10th "	.21	.34	2.78	.77	4.83	4.8	13.73
14th "	.19	.31	2.68	.75	4.74	4.6	13.27
21st "	.20	.34	2.54	.74	4.78	4.5	13.10
28th "	.29	.27	2.55	.74	4.57	4.6	13.02

[1] Average figures for group of cows made up of 3 Holsteins; 2 Guernseys; 1 Ayrshire; 1 Jersey.

[2] Calculated sum of the constituents.

INFLUENCE OF BREED ON CURD TENSION

There is a good deal of interest in the influence of the breed on curd tension. Hill (9) reported the first results from herds tested in Utah, with only a limited number of cows of the Guernsey and Ayrshire breeds represented. The Ayrshire breed association[3] since has published figures showing a higher percentage of soft curd cows for that breed. In view of the marked correlation between protein and curd tension, it would be expected that in

[3] The Ayrshire Digest, 18: 3–6, 1932.

curd tension distribution the different breeds would rank approximately in the same order as the average casein or total protein content of their respective milks. Under these conditions one would expect the percentage of soft curd cows to decrease in the different breeds in approximately the following order: Holstein, Ayrshire, Guernsey and Jersey.

The results of a considerable number of determinations run on the milk of these breeds during the past two years show this to be the case. These figures are given in Table 5 and represent values obtained for the cows in the herd of the Kansas Agricultural Experiment Station and samples submitted by dairymen in the state. The number of cows tested in the Jersey breed is not large, but since the results for this breed agree fairly well with the distribution observed by Hill (9), it is felt that they are sufficiently representative for the purposes of this comparison. If a curd tension of 30 grams or below is considered as the dividing line for soft curd milk, it will be seen that the Holstein breed has an appreciably higher percentage of cows in this classification than the other dairy breeds, followed by the Ayrshire, Guernsey and Jersey breeds in the order listed. In general, Holstein milk shows a lower average curd tension and the percentage of cows testing over 80 grams is substantially lower than for any of the other breeds. Considering the similarity in composition, it is rather surprising that the Guernsey breed shows a significantly higher percentage distribution in the lower classifications than the Jersey breed.

TABLE 5

Percentage distribution of curd tension by breeds

BREED	NO. OF COWS TESTED	NO. OF TESTS RUN	CURD TENSION—GRAMS					
			Below 30	31–40	41–60	61–80	81–100	Over 100
			(Percentage of cows)					
Ayrshire	55	319	10.9	20.1	37.6	19.2	10.3	1.9
Guernsey	70	221	8.1	15.4	36.2	23.1	12.3	4.9
Holstein	157	442	18.5	22.9	35.1	18.5	4.5	0.5
Jersey	21	172	2.9	4.1	16.9	27.9	24.4	23.8

SUMMARY AND CONCLUSIONS

Using a modification of the Hill technique for determining curd tension, a study was made of a number of the principal factors influencing the curd tension of milk under normal conditions.

The daily variation in cows producing milk ranging from soft to medium curd tension was observed to be significant in the selection of cows for the production of soft curd milk.

Stage of lactation was found to have a considerable influence on the curd tension. Following a rapid decline in the first month, the lowest values were obtained in the second and third months, followed by a gradual and significant increase to the end of the lactation. This held true for producers of soft curd milk, average values being obtained in the tenth month that were approximately 100 per cent higher than in the second month of the lactation. The influence of daily variation and stage of lactation emphasize the need for regular tests if milk of strictly soft curd character is to be produced.

Data are presented on the important changes in the composition of milk for approximately the first month following parturition. The relation of these changes to the curd tension behavior are discussed. A correlation of $0.76 \pm .04$ was obtained betwen curd tension and the total protein content of milks on 58 samples of milk from the experiment station herd.

The breed was found to be one of the most important factors influencing curd tension. In the present study the breeds ranked in the following order of decreasing importance as producers of soft curd milk—Holstein, Ayrshire, Guernsey and Jersey.

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RENNET TEST FOR THE DETECTION OF MASTITIS*

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The observation that milk from cows with mastitis does not coagulate readily, or not at all, with rennet is not new. Apparently Schern (1, 2, 3, 4, 5) was the first investigator to describe and employ a standardized technic for retarded rennet coagulation for the diagnosis of mastitis. Laue (6) experimented with Schern's test and reported it to be unreliable. Orla-Jensen (7) suggested that rennet would be helpful in determining if milk has been drawn from a diseased udder, but he apparently never applied the suggestion in a practical way. Rosell (8) stated that the capacity of mastitis milk for coagulation by rennet is greatly diminished because of the decrease and changes in casein, but he has not published detailed directions for the use of rennet for such purpose. The test to be described in this paper was first demonstrated before the Dairy Manufacturers' Short Course (9) at the University of Wisconsin.

PRELIMINARY STATEMENT

The test with rennet is based upon the principle that the enzyme rennin readily causes the casein fraction of normal milk to coagulate. Abnormal or mastitis milk has less casein and calcium than normal milk, so does not coagulate so quickly, if at all. Another factor responsible for failure of mastitis milk to coagulate is the increased pH.

For most reliable results with the rennet method of testing for mastitis individual quarter samples of fresh milk should be taken. Mastitis milk, even when diluted so low as 1 : 3 with normal milk, may not react with this test on account of the dilution factor. Likewise a composite milk sample from the four quarters of the cow, or mixed samples from cans or bottles are not suitable. As the first drawn milk is more likely to give a reaction when this method is used, it is preferable to middle milk or strippings.

An especially desirable feature of the rennet test is that no expensive laboratory equipment is required. However, if an incubator is available to hold the temperature at a given point, more uniform results can be secured. A supply of test tubes of 10 or 15 cc. capacity and one 1-cc. pipette graduated in 1/10ths is all the glassware needed. In place of the pipette a dropper with rubber nipple adjusted to deliver the required amount of rennet solution may be used. Suitable racks for holding the test tubes are desirable. Either an interval timer, or a stop watch, will be found helpful.

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* Published with the permission of the Director of the Wisconsin Agricultural Experiment Station.

TECHNIC OF THE RENNET TEST

The rennet testing solution consists of one part fresh, commercially prepared fluid rennet extract mixed with 50 parts distilled water. A fresh solution should be made whenever tests are to be run as the diluted extract rapidly loses its coagulating power. Five cubic centimeters of milk from the quarter to be tested are drawn directly into a test tube, which has a mark indicating the height to which the tube should be filled. To this milk sample is added 0.1 cc. of the diluted rennet, making a final dilution of 1 : 2500. This combination is mixed by inverting the tube and allowed to stand one hour at room temperature ranging from 72° to 82° F. Normal milk will coagulate within this period. Samples that fail to coagulate are abnormal. Coagulation is determined by tilting the tubes to a horizontal position. The test is used as presumptive evidence of mastitis. Degrees of the disease can be detected by noting the results at 15-minute intervals.

Workers should appreciate that mastitis milk not only has less casein, but also a lowered concentration of sugar, and calcium and potassium salts, all of which are substances elaborated by the milk-secreting cells of the udder. On the other hand, mastitis milk has a higher water content and contains more albumin, chlorides, catalase and leucocytes, all of which are substances derived directly from the blood and are increased as a result of the inflammatory process. Furthermore, such milk usually is more alkaline in reaction than normal milk and its nutritional value is lower. Practically it is interesting to note that the value of mastitis milk for cheese manufacture is strikingly reduced. The chief difference according to Prof. W. V. Price, of Wisconsin, is in the nature of the curd, which is decidedly soft and correspondingly more difficult to handle. Another drawback is the loss of more butterfat during the manufacturing process, resulting in less economical cheese production.

EXPERIMENTAL

A comparison of the results of testing with rennet 20 samples of milk from five different cows is presented in Table I. These particular cows were selected from the thousands upon which records have been secured because they represent different degrees of mastitis as revealed by the rennet test. By way of explanation it should be stated that the numbers under each cow refer to the quarters: 1 is right front, 2 right rear, 3 left rear, and 4 left front. The + sign indicates no coagulation or a reaction; − coagulation or no reaction; ? partial coagulation or an incomplete reaction. The reactions were recorded at 15-minute intervals for one hour in order to determine the rapidity of the reaction, which reflects the degree of infection. For instance, by means of the rennet test it was shown that Cow 1 is mastitis-free; Cow 2 has the disease in quarter 3; Cow 3 in quarters 2 and 4; Cow 4 in quarters 1 and 2, and possibly in 3; Cow 5 in all quarters.

TABLE I

Comparison of results of the Rennet test with other methods

TEMP. 78° F.	COW 1				COW 2				COW 3				COW 4				COW 5			
Method of testing for mastitis	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Rennet after 15 min.	+	+	?	−	+	+	+	−	+	+	?	+	+	+	+	+	+	+	+	+
" " 30 "	−	?	−	−	−	−	+	−	−	+	−	+	+	+	+	+	+	+	+	+
" " 45 "	−	−	−	−	−	−	+	−	−	+	−	+	+	+	?	−	+	+	+	+
" " 60 "	−	−	−	−	−	−	+	−	−	+	−	?	+	+	−	−	+	+	+	+
Brom cresol purple	−	+	−	−	−	−	+	−	−	+	−	?	+	+	−	−	+	+	+	+
Chlorides	−	+	−	−	−	−	+	−	?	+	−	+	+	+	?	−	+	+	+	+
Catalase	−	−	−	−	?	−	+	−	−	+	−	+	+	+	−	−	+	+	+	+
Bacteriological	+	−	−	−	−	−	+	−	−	+	−	+	+	−	+	+	+	+	+	+
Summarized results	−	?	−	−	−	−	+	−	−	+	−	+	+	+	?	−	+	+	+	+

There are also presented for comparison in Table I results secured from testing for mastitis the same series of milk samples by several other diagnostic methods. Although the results do not check exactly, a general relation exists between them. One reason for the variations is that the methods are based on different factors, so are not entirely comparable. This will serve to impress the important point that reliance should not be placed on the results of one test by any method. A more dependable diagnosis can be secured by basing conclusions on the combined or summarized results from several methods of testing, as has been done in the last line of the table. Retests at a later date are desirable in cases of questionable reactions to establish the diagnosis beyond reasonable doubt, as well as to detect new cases. In practice, wider variations will be found than are shown in the results compiled in Table I which it will be recalled were from a selected group of cows. Also values will vary from week to week with the same cow due to changes occurring in composition of the milk.

TABLE II

Comparison of the value of results by combined and single methods

METHOD OF TESTING FOR MASTITIS	SAMPLES ABNORMAL		SAMPLES NORMAL	
	No.	Per cent	No.	Per cent
Combination of all methods	67	21	247	79
Rennet	62	20	252	80
Brom cresol purple	79	25	235	75
Chlorides	98	31	216	69
Catalase*	64	20	250	80
Bacteriological*	42	19	182	81

* The tests for catalase and bacteriological examinations were made by cooperators in the laboratories of the Department of Agricultural Bacteriology. To avoid complications borderline or suspicious reactions are classified as either positive or negative depending on the degree of reaction.

In Table II a comparison is made of the value of a combination of the various methods of testing employed in our experimental work with that of each method. It shows that of the 314 quarter samples of milk tested 67 or 21 per cent were found to be abnormal and 247 or 79 per cent normal. By the rennet method the percentages were 20 and 80 respectively. A higher percentage of reactions was secured with brom cresol purple and the silver nitrate-potassium chromate method for chlorides as described by Hayden (10). This may be accounted for, in part at least, by the concentration of the insoluble and soluble milk salts in some of the samples, which were from cows nearing the end of their lactation period. The results with the catalase method compare favorably to those with rennet, but as they depend upon the number of leucocytes, or their products of disintegration, they also are somewhat influenced by the stage of lactation. The results of the bacteriological examination are not strictly comparable with any of the other methods because they are based on the counts for streptococci rather than on the composition of the milk.

CONCLUSIONS

From this preliminary study it is concluded that (1) the rennet method of testing milk for mastitis is as reliable as any other with which it was compared; (2) all methods have limitations; (3) a more dependable diagnosis can be made if milk samples are tested by two or more methods, *i.e.*, a combination of results by different methods furnishes more reliable information; (4) one series of tests on a herd does not furnish all needed information; (5) the composition of milk from the same quarters of the same cow varies from week to week.

SUMMARY

A new test with fresh commercial fluid rennet for the detection of mastitis is described. It is believed that this method of testing, because of its simplicity and cheapness, may have practical use. Comparisons of the results secured by the rennet method with those secured by other methods are presented.

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BUTTERFAT AND TOTAL SOLIDS IN NEW ENGLAND FARMERS' MILK AS DELIVERED TO PROCESSING PLANTS

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INTRODUCTION

Milk is the whole, fresh, lacteal secretion, colostrum free, obtained by the complete milking of one or more healthy cows, properly fed and kept, and which after production is not altered in composition, adulterated, or processed.

The State of Massachusetts requires that milk shall contain not less than 3.35 per cent of butter fat and 12.00 per cent of total solids, a standard which is inconsistent in its relation of fat to solids-not-fat. In order to meet the requirements it became the practice of milk distributors to standardize solids not fat up to the legal requirements by the addition of condensed skim milk, the amount used varying with the season of the year. Legally one may raise the total solids of milk only by the addition of very rich milk or cream.

If one reviews the standards of our other states, we find whereas certain states set a minimum fat standard, others either fail to recognize the value of a standard for solids-not-fat and total solids, or have such standards that are inconsistent in their relation of fat to solids-not-fat.

In the scoring of solids-not-fat in milk we find that the United States Department of Agriculture has adopted the following standards:

	Points		*Points*
8.7 per cent and over	15	8.2 per cent	5
8.6 " " " "	13	8.1 " "	3
8.5 " " " "	11	8.0 " "	1
8.4 " " " "	9	Less than 8 per cent	0
8.3 " " " "	7		

Note:—When the percentage of solids-not-fat is less than the local limit the score should be 0.

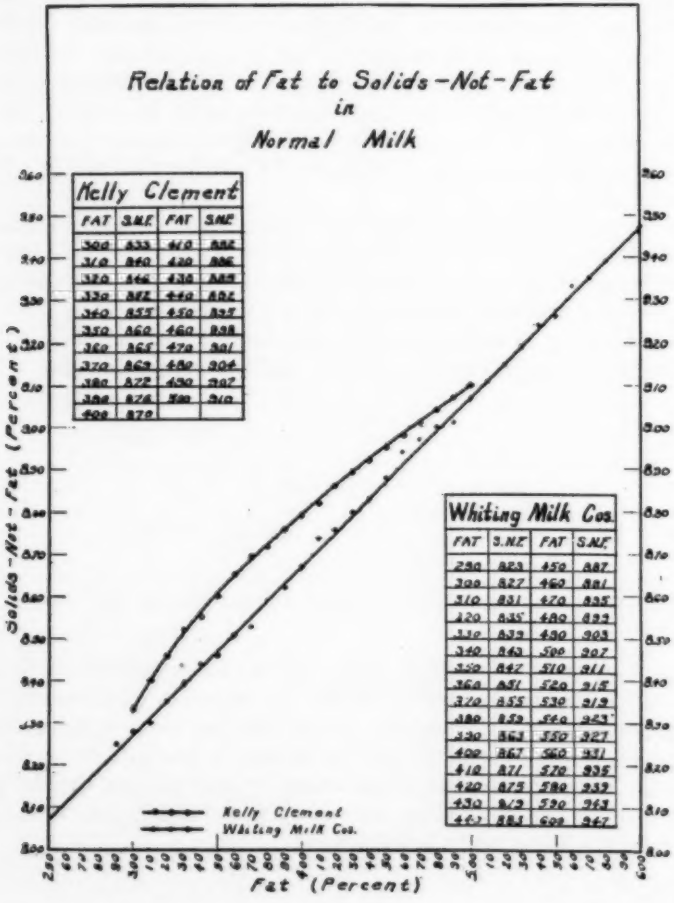
The dairy industry has long recognized the importance of the Babcock test and has regularly provided for the determination of butter fat value in making payment to producers. It has however failed to recognize the importance of solids-not-fat in milk and to provide for the determination of abnormal, watered, skimmed, added cream, or milk of poor quality, all of which results in an unbalanced milk costly to the distributor, who may be subjected to prosecution by health officers.

The president of our company, Mr. Joseph Willmann, knowing the value of solids-not-fat in milk requested that practical provisions be made for the determination of its content in each of our producer's milk at least once weekly.

This work has resulted in a marked improvement of quality and if nationally adopted it would be only a question of time when our general standard of quality would be materially improved and consumption increased.

METHODS

During the past sixteen months over 100,000 samples of Massachusetts, New Hampshire, Vermont, Maine, Connecticut and Rhode Island producers'



milk have been analyzed for fat and total solids. These samples were composed of individual cows, of herds, and of mixed milks, representing all breeds and grades of cows.

The method analysis used was as follows:

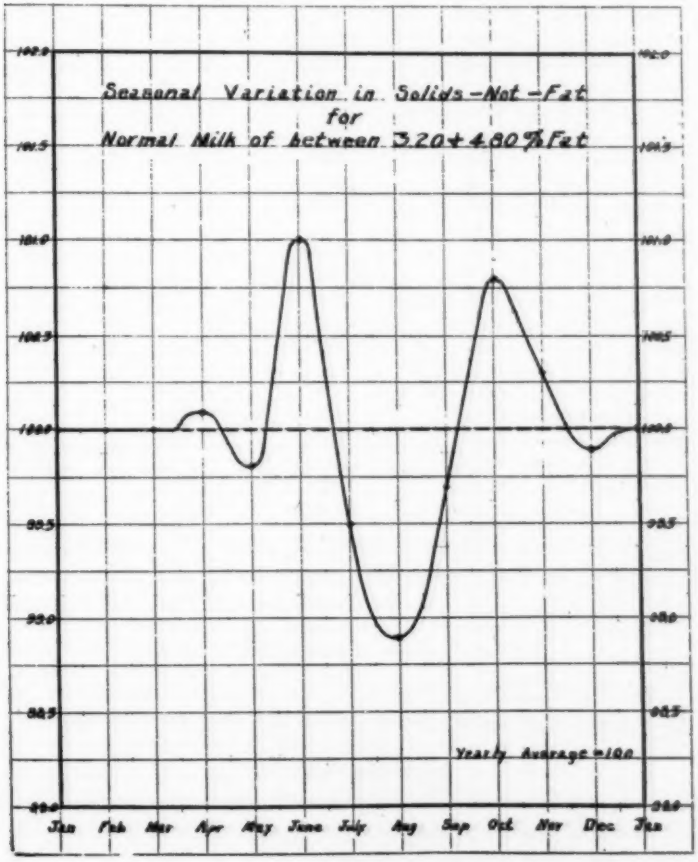
Total Solids—Quickly weigh out 5 grams of a well mixed sample into a tared, flat-bottom dish, and evaporate over a bath of boiling water until it ceases to lose weight (2 hours). Cool at room temperature in a dessicator for five minutes and quickly weigh the residue.

Fat—By the Babcock and Mojonnier methods.

Solids-not-Fat—Obtained by difference.

RESULTS

Chart 1 has been prepared from Table 1 to summarize the findings of



our analyses and represents in excess of 100,000 samples of New England farmers' milk. The work of Kelly and Clement[1] is also given.

In comparing our chart with that of Kelly and Clement, we find our New England milk averaging 0.11 per cent lower in solids-not-fat. Solids-not-fat consistently increase by 0.04 per cent with each increase of 0.1 per cent in fat.

TABLE 1

Relation of fat to other solids in producers milk New England States

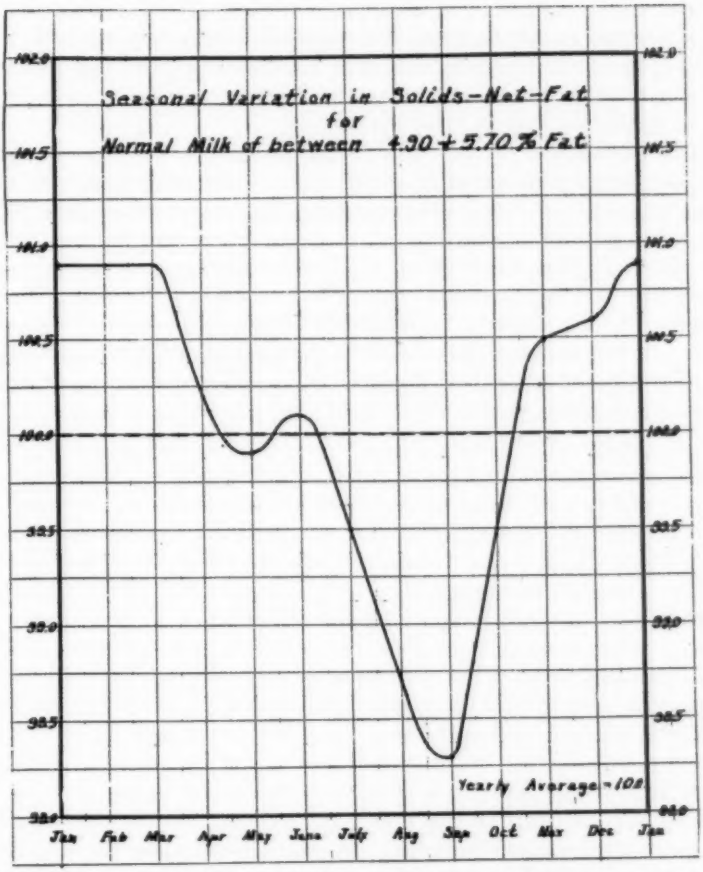
FAT	SOLIDS-NOT-FAT	TOTAL SOLIDS	RATIO OF FAT TO SOLIDS-NOT-FAT	SOLIDS-NOT-FAT IN SERUM
2.80	8.19	10.99	1: 2.93	8.42
2.90	8.23	11.13	1: 2.84	8.47
3.00	8.27	11.27	1: 2.76	8.52
3.10	8.31	11.41	1: 2.68	8.57
3.20	8.35	11.55	1: 2.60	8.62
3.30	8.39	11.69	1: 2.54	8.67
3.40	8.43	11.83	1: 2.48	8.73
3.50	8.47	11.97	1: 2.42	8.78
3.60	8.51	12.11	1: 2.36	8.83
3.70	8.55	12.25	1: 2.31	8.88
3.80	8.59	12.39	1: 2.26	8.93
3.90	8.63	12.53	1: 2.21	8.98
4.00	8.67	12.67	1: 2.17	9.04
4.10	8.71	12.81	1: 2.12	9.09
4.20	8.75	12.95	1: 2.08	9.14
4.30	8.79	13.09	1: 2.04	9.19
4.40	8.83	13.23	1: 2.00	9.24
4.50	8.87	13.37	1: 1.97	9.29
4.60	8.91	13.51	1: 1.93	9.34
4.70	8.95	13.65	1: 1.90	9.40
4.80	8.99	13.79	1: 1.87	9.45
4.90	9.03	13.93	1: 1.84	9.50
5.00	9.07	14.07	1: 1.82	9.56
5.10	9.11	14.21	1: 1.79	9.60
5.20	9.15	14.35	1: 1.76	9.66
5.30	9.19	14.49	1: 1.73	9.71
5.40	9.23	14.63	1: 1.71	9.75
5.50	9.27	14.77	1: 1.69	9.82
5.60	9.31	14.91	1: 1.66	9.88
5.70	9.35	15.05	1: 1.64	9.91
5.80	9.39	15.19	1: 1.62	9.97
5.90	9.43	15.33	1: 1.60	10.00
6.00	9.47	15.47	1: 1.58	10.01

Massachusetts legal requirements not less than 3.35 fat—12.00 total solids.

SEASONAL VARIATION

The seasonable variation in solids-not-fat for normal milks are shown in Charts 2 and 3, the average analyses being taken as 100:

[1] Market milk, John Wiley and Sons, 1923.


Seasonal Variation in Solids-Not-Fat
for
Normal Milk of between 4.90 + 5.70 % Fat
Yearly Average = 102
Jan Feb Mar Apr May June July Aug Sep Oct Nov Dec Jan

It will be noted that the variations in solids-not-fat for normal milk of between 3.20 and 4.80 per cent fat is not so marked and the period of low and high solids-not-fat do not occur in the same periods as normal milk of between 4.90 and 5.70 per cent fat.

DISCUSSIONS AND CONCLUSIONS

The analyses of more than 100,000 samples of milk delivered to plants in New England show that for each increase of 0.1 per cent in butterfat there is a uniform increase of 0.04 per cent in milk solids-not-fat. There is a small seasonal variation in this relationship.

The percentage of solids-not-fat found in this investigation was 0.11 per cent less than the average analysis reported by Kelly and Clement.

Milk containing just enough butter fat to be legal in Massachusetts was below standard in solids-not-fat. Since one can legally raise the milk solids only by the addition of very rich milk it is obvious that these solids are valuable not only from a nutritional and flavor standpoint but also in the cost of producing milk of legal composition.

It is believed that the time may be ripe to establish two legal standards for milk; one to cover the milk sold by producers to dealers, and the other to cover the milk as sold to the consumer. The milk standard for production would permit the majority of all normal herd milk to come within that standard. The standard for milk sold to consumers should require a composition that would ensure a good flavor to assist in increasing the consumption of milk solids by a greater total consumption and by more milk solids per unit volume. This would be brought about by standardization of milk above the production standard by the addition of cream to a four or a four and a half per cent butter fat content.

THE EFFECT OF INTRAVENOUS INJECTIONS OF SUGAR UPON THE LACTATING BOVINE*

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Having satisfactorily demonstrated that artificially produced hypoglucemia results in a lowering of the lactose content of cows' milk, it was considered desirable to investigate the possibility of an artificially induced hyperglucemia as a means of increasing the lactose content.

HISTORICAL

Effect of sugar injections on milk secretion

Piantoni (7) was the first worker to study the effect of sugar injections upon milk secretion. Using milch goats as the experimental animals, he found that only the mono- and di-saccharides, when hypodermically injected, had any effect on milk secretion. Both mono- and di-saccharides injected in small doses of 1 gram daily resulted in an increased milk secretion but with no change in the lactose percentage. Large doses of sugar, however, decreased both the milk yield and its lactose content and if repeated daily for several weeks diminished the yield as much as fifty per cent.

Sammartino (8) applied Piantoni's discovery to his study with lactating women. He was able to triple the milk secretion by small daily injections of about 5 cc. of 1.5 per cent sucrose in cases where the women secreted too little for their babies, and was able to diminish milk secretion by injecting 5 cc. of 40 per cent sucrose daily in cases where it was advisable to check secretion.

Monaco, Nazari and Romolotti (5) were likewise able to increase the milk secretion in cows already producing a large quantity of milk by means of subcutaneous injections of sucrose. Campus (2) also reports an increase in the quantity of milk produced by three ewes, following ten daily subcutaneous injections of 1 cc. of a 50 per cent sucrose solution.

Effect of sugar injections on the percentage of lactose in the milk

Nitzescu (6) made intravenous injections of glucose, levulose, galactose, maltose and sucrose into milch goats at the rate of 1 to 2 grams per kilo live weight. He found that only the mono-saccharides and maltose can be used

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* The data in this paper are taken mainly from a thesis presented by W. R. Brown in partial fulfillment for the Ph.D. degree and published with the approval of the Director as Paper No. 1383.

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for sugar synthesis and observed no change in the quantity of milk secreted or in its fat content, but a slight change in the lactose content. He concluded that the mammary gland retains an autonomy as regards lactose secretion, which is produced in a very constant manner in spite of wide blood sugar variations.

Macchiarulo (4) says he has produced hyperglucemia in a goat by the injection of 200 cc. of 15 per cent glucose. He asserts he obtained an increase of lactose in the milk but fails to substantiate his claim with numerical data

THE PROBLEM

Several workers have made intravenous sugar injections into various species of lactating mammals with the object of determining the effect upon the quantity of milk produced.

Nitzescu (6) alone reports a slight increase in the lactose content following sugar injections into milch goats. It was therefore considered that a further study of this subject, using the lactating bovine, would be of value.

Successive experiments using glucose, glutose, fructose, and lactose were planned in which repeated injections of the particular sugar would be made in an attempt to produce a hyperglucemia for several hours so that a marked effect upon the sugar content of the milk would be possible.

EXPERIMENTAL

Methods

All samples were collected, and the sugar determinations carried out in the manner previously described.

Intravenous injections

The intravenous sugar injections were made into the jugular vein with a 100 cc. hypodermic syringe, immediately after the collection of a blood sample and without the removal of the needle. Sterilized isotonic sugar solutions were used in all the experiments.

Experiments

The first three experiments consisted of the hourly milking out of cows and the periodic intravenous injection of sugar solutions. In these initial experiments only small amounts of sugar were used.

In Experiment 1, cow 544 was milked at hourly intervals from 6:30 A.M. until 11:30 A.M. when an initial dose of 3.5 grams of glutose was injected into the jugular vein. A second dose of similar size was injected at 2:10 P.M. while a third and last dose of 3.5 grams of the same sugar was given at 3:15 P.M.

The same cow was used in Experiment 2 which was of similar nature to Experiment 1 with the exception that the initial dose of glutose was followed by a single 7.0-grams dose at 1:00 P.M.

The results of these two experiments are graphically illustrated in the upper half of Figure 1 accompanied by the curve obtained when the same cow was milked at hourly intervals without sugar injections.

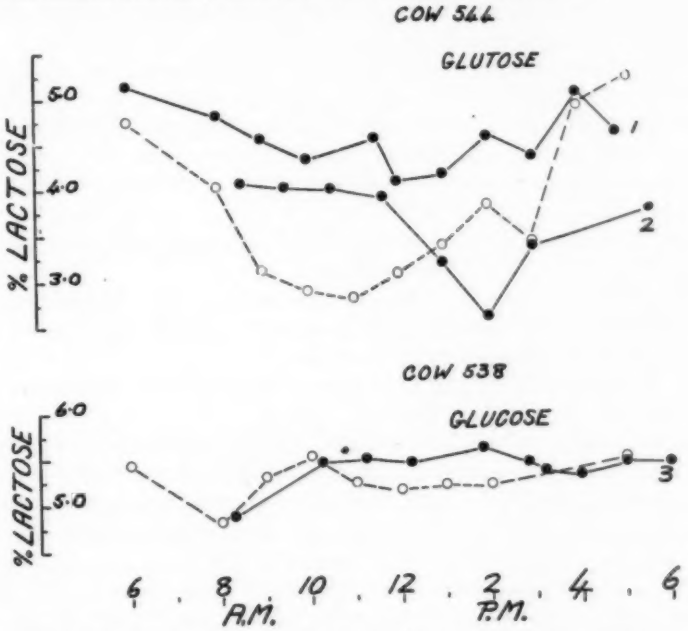

FIG. 1. Effect of intravenous injections of glucose and glutose upon the lactose content of milk.

Protocol.
Cow 544. Curve 1.
11:30 A.M. 3.5 gms. glutose injected.
2:10 P.M. 3.5 gms. " "
3:15 P.M. 3.5 gms. " "

Protocol.
Cow 544. Curve 2.
11:40 A.M. 3.5 gms. glutose injected.
1:00 P.M. 7.0 gms. " "

Protocol.
Cow 538.
8:20 A.M. 8 gms. glucose injected.
10:10 A.M. 8 gms. " "
11:10 A.M. 12 gms. " "
12:10 P.M. 16 gms. " "
2:10 P.M. 20 gms. " "

--------o-------- Normal
———— ● ———— Sugar injected

No abnormal effects noted in any of the above experiments except a slight temperature rise in case of cow 538.

As the lactose showed no pronounced increase following the glutose injections, it was decided to use the common sugar glucose instead and to increase the frequency of injections.

In Experiment 3, cow 538 was milked hourly from 6:00 A.M. and intravenous glucose injections were made at 8:20 A.M., 10:10 A.M., 11:10 A.M., 12:10 P.M. and 2:10 P.M., a total amount of 64 grams of glucose being injected. The protocol of this experiment accompanies the results obtained which are illustrated in the lower half of Figure 1.

Failing to produce any marked changes in the secretion during the first three experiments, it was decided to extend the scope of the work by the additional collection of blood samples in the hope that the blood sugar curves would facilitate in the interpretation of the results.

Glucose, fructose and lactose were the sugars used in these additional experiments.

In Experiment 4, cow 538 was injected four times over a period of three hours with a total of 217 grams of glucose.

Experiment 5 consisted of two intravenous injections totaling 35 grams of fructose into cow 167 while in Experiment 6, cow 165 was injected four times with a total of 34 grams of fructose.

In Experiment 7, 35 grams of lactose were injected at one time into cow 167.

Because every intravenous injection, with the sole exception of the lactose injected in Experiment 7, failed to produce but a very temporary hyperglucemic condition, a slow injection was attempted in Experiment 8 in hope that constant high level of glucose could be maintained. For this purpose a board was wired to the horns of cow 538 and one ear strapped to it. The fastened ear was anesthetized with novocain and a fine hypodermic needle then inserted into an ear vein. The glucose solution was forced by air pressure from a two litre Winchester bottle through a rubber tube into the needle. This injection was continued only for thirty minutes, as the cow became too excited by the manipulation. The injection was carried out between 10:30 A.M. and 11:00 A.M., and during this time a total of 30 grams of glucose was injected.

At 11:30 A.M., 18.75 grams of glucose were injected intravenously in the ordinary way and this was followed by a final injection of 7.5 grams of glucose at 1:00 P.M. The results of Experiments 4, 5, 6, 7, and 8 are graphically illustrated in Figure 2, curves 2, 3, 4, 5, and 1 respectively, and are also accompanied by the protocols.

DISCUSSION OF RESULTS

Figure 1 illustrates graphically the hourly trend of milk sugar following the intravenous injection of glutose (curves 1 and 2) and the intraven-

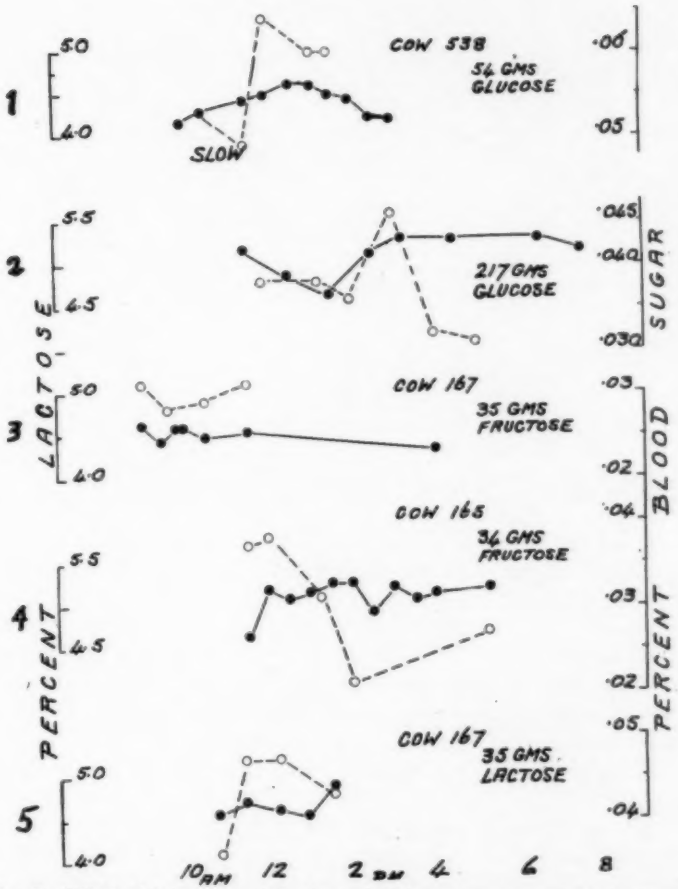

FIG. 2. Effect of intravenous injections of different sugars upon blood sugar level and the lactose content of milk.

——— ● ——— Lactose
- - - - - - - o - - - - - - - Blood sugar

Protocol. Curve 1.
Cow 538.
10: 30 to 11: 00 A.M. Slow injection of 30 gms. glucose.
11: 30 A.M., 18.75 gms. glucose injected.
1: 00 P.M., 7.5 gms. " "
10: 30 A.M. Injected 2% novocain into ear and made several attempts to carry out a continuous slow injection of the glucose solution. The continuous manipulation around the animal's head, however, caused so much nervousness that the slow injection was discontinued. Immediately following the 11: 30 A.M. glucose injection, a twitching started of the muscles over the whole of the body. This vigorous twitching continued for about 30 minutes, then gradually subsided. The animal was apparently normal when the 1: 00 P. M. glucose injection was made, and except for a rise in temperature showed no further abnormal symptoms.

Protocol. Curve 2.
Cow 538.
11: 45 A.M., 21.0 gms. glucose injected.
1: 10 P.M., 59.5 gms. " "
2: 00 P.M., 45.5 gms. " "
3: 00 P.M., 91.0 gms. " "
No abnormal symptoms noticed.

Protocol. Curve 3.
Cow 167.
9: 10 A.M., 17.5 gms. fructose injected.
9: 50 A.M., 17.5 gms. " "
No abnormal symptoms noticed.

Protocol. Curve 4.
Cow 165.
11: 40 A.M., 7.5 gms. fructose injected.
12: 00 M., 7.5 gms. " "
1: 30 P.M., 11.5 gms. " "
2: 00 P.M., 7.5 gms. " "
No abnormal symptoms noticed.

Protocol. Curve 5.
Cow 167.
10: 50 A.M., 35 gms. lactose injected.
No abnormal symptoms noticed.

ous injection of glucose (curve 3), as well as the milk sugar curves of the normal animals obtained within a day or so of the experiment.

It was thought that if a sugar injection actually resulted in an increased lactose secretion the lactose would be not only higher but also more constant in the successive hourly samples. While the curves obtained are higher than the normals, the marked fluctuations of all of them indicate that the apparent increase may be only accidental. No blood sugar determinations were run in these experiments, so further injections were made to see whether the blood sugar picture would help in the interpretation of results.

In the experiments graphed in Figure 2, both the blood and milk sugar fluctuations were determined. Some of these experiments are very short, as they were preliminary runs, the results of which were not considered promising enough to warrant more extended studies.

The blood sugar in curve 1 shows a marked decrease from 10: 30 A.M. to 11: 30 A.M., during which time 30 grams of glucose were slowly injected into an ear vein. It was at this low point that the instantaneous injection of 18.75 grams of glucose into the jugular vein apparently resulted in the tremors recorded in the protocol. Following this injection, hyperglucemia occurred.

In curve 2 the injections of glucose first caused a slight hypoglucemia, then a marked hyperglucemia, and finally a very pronounced hypoglucemia.

In curve 3 the first injection of fructose resulted first in a slight hypoglucemia, while the second injection had no immediate apparent result.

In curve 4, however, the fructose injection caused a very pronounced hypoglucemia of several hours' duration. The lactose injection depicted in curve 5 was the only one which resulted in a pronounced hyperglucemia.

The unexpected behavior of the blood sugar following intravenous sugar injections suggests that the apparent increase in the lactose secreted following some of the injections may be, as already mentioned, insignificant or due to some outside factor.

It has long been known that sugar ingestion in human beings results in first hyperglucemia followed by a pronounced hypoglucemia, then a gradual rise back to the normal level. Harrop and Benedict (3) who noted the above phenomenon offered the following tentative explanation: "It must be supposed that regulation of the output of pancreatic hormone is normally in some way governed by the amount of material awaiting metabolism. Ingestion of food high in carbohydrates should call out amounts of insulin adequate to metabolic requirements, and it would be expected that the effect of carbohydrate metabolism due to the natural hormone upon serum concentration would be similar to that produced by injection of the product prepared artificially." This suggestion seems to explain the results here reported, although the milk sugar curves do not follow the same downward trend that was noticed following the injection of large doses of insulin. A careful search of the literature has revealed a similar hypoglucemia following the injection of glucose in normal human beings, when Thalhimer, Raine, Perry and Buttles (9) report that slow intravenous injections of 10 per cent glucose caused a rather severe hypoglucemia with results similar to a moderately severe insulin shock. The hypoglucemic effect produced by the intravenous injection of sugar solutions may be the cause of some of the results reported by other workers. While no adequate explanation can be offered to satisfy the already mentioned increased milk production obtained (by Piantoni, Sammartino, Monaco and coworkers, and Campus) upon the injection of small amounts of sugar, the decrease in volume due to the injections of large amounts of sugar as noted by Piantoni and Sammartino may be the result of a daily artificially induced hypoglucemic condition. In support of this suggestion there is the experimental work of Bucciardi (1) who found 5 or 6 successive insulin injections caused cessation of lactation in ewes, and our own experience in which cow 143 stopped lactating after its third insulin experiment.

CONCLUSIONS

Apart from the interesting hypoglucemic effects noted, the value of intravenous sugar injections in a study of lactose synthesis seems to be of very doubtful value, as the apparent slight success of Nitzescu might be easily caused by a hitherto unsuspected normal variation in the milk sugar of the goat of the same magnitude as we have found to frequently occur in

the lactating bovine. The reported success of Macchiarulo, on the other hand, is not supported by numerical data so must be neglected.

SUMMARY

1. Intravenous injections of glucose and fructose may result in a hypoglucemic condition in the bovine.

2. An intravenous injection of lactose resulted in a marked hyperglucemia.

3. A suggestion is offered to account for the decrease in milk secretion noted by many workers following daily injections of large amounts of sugar.

4. The intravenous injections of sugar are considered of doubtful value in the study of lactose synthesis.

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BITTER FLAVOR IN CHEDDAR CHEESE

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Bitter flavor is frequently found in Cheddar cheese. It may be only slightly apparent, or may actually make the product unpalatable. This defect has been observed by numerous workers, some of whom have been able to show specific causes for it. The typical citations which follow illustrate the variety of causes which have been indicated.

Publow (1) attributed bitter flavor in cheese to undesirable organisms, dirty cows, old starters and unclean or rusty milk cans. Allen (2) found it in cheese made from milk of inferior quality. He observed that alkali-forming and protein-splitting bacteria were present in larger numbers in the inferior milk than in clean milk, but that there was a marked tendency for the development of these organisms in the cheese made from clean milk, rather than in that made from inferior milk.

Some investigators believe that the defect can be traced to certain microorganisms. Hucker and Marquardt (3) were able to associate bitter flavor with *S. paracitrovorus* and certain strains of acid-proteolytic cocci. *S. paracitrovorus* in raw milk showed slightly bitter flavors after twelve weeks, which did not occur in cheese made from identical milk with Hansen's culture. In pasteurized milk *S. paracitrovorus* also showed slightly bitter flavor. Acid-proteolytic cocci gave a decidedly bitter flavor in two to three weeks which eventually made the cheese unpalatable. Riddet, Valentine, and Whitehead (4) described a bitter flavor which they produced by using a particular starter culture which had become a rather weak acid producer in the cheese vat. Bitter or metallic flavor was also found in the starter itself, as well as in the cheese. They added, however, that not all "slow" or bitter-flavored starters produce bitter cheese.

Riddet, Valentine and Whitehead (4), in reviewing the possible causes of bitter, flavor, observed that cheese made in their laboratory from normal milk, not overheated during pasteurization, frequently developed bitterness at an intermediate stage in the ripening process. The bitterness sometimes disappeared subsequently and the Cheddar flavor developed in an otherwise normal manner. This cycle had previously been observed by Allen (2) who noticed that bitterness in cheese made from inferior milk developed after four weeks and disappeared after sixteen weeks of curing. Kelly (5) commented on this sequence by stating that "many investigators who have studied the ripening processes in cheese have considered that the peptone

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stage of the decomposition of protein gave a characteristic bitter flavor to the curing cheese." Like the other workers, he indicated that, after 6 to 8 weeks of curing at temperatures higher than 10° C., a definite bitter stage was passed through before the final characteristic, mellow flavor was developed.

Pasteurized milk is frequently associated with bitter flavors, although those acquainted with the procedure usually attribute the defect to high temperatures of heating or to improper methods of curd making. Kneutinger (6) believed that excess whey in cheese made from pasteurized milk caused bitter flavor. Phillips (7) found the defect when milk was flash pasteurized to 177° F. Moir (8) noticed bitter flavors in cheese made from milk heated to 185° F.

In this laboratory the development of bitter flavors has been associated for the past few years with the use of certain starters and with the control of acidity during the curd making process. The defect seemed more apt to occur when the cheese showed a slight excess of acid development. It is understood that this relation has also been observed by workers in the laboratories of the United States Department of Agriculture, Bureau of Dairy Industry (9).

Phillips (10) has reported preliminary experimental results of work completed in this laboratory, which seem to show that development of acidities greater than pH 5.00 at 4 days after making, may contribute to the production of bitter flavor in Cheddar cheese.

EXPERIMENTAL

The manufacturing records from 178 lots of experimental cheese which were made in 1932 have been selected to study the relationship between bitter flavors and acidity of cheese. Commercial starter cultures were used and all lots of cheese were made from raw milk of average quality. Normal variations of the curd making process such as acidity of milk, size of cubes, temperature of cooking and the like, are represented in these data. No water, salts, except sodium chloride, or any other substances were added to the milk, whey or curd.

The methods used in measuring acidity in the cheese have been described elsewhere (11).

The criticisms of the cheese were made by competent judges who examined them two or three times during a curing period of approximately one year. The cheese were not scored frequently enough to indicate exactly when the bitter flavors first appeared or when they disappeared, as some undoubtedly did.

The quality of the cheese is indicated by numbers ranging from 1 to 6. The interpretation of these values may be briefly summarized as follows:

1—Excellent, no criticism.

2—Desirable.

3—Satisfactory commercial quality.
4—Objectionable.
5—Very undesirable.
6—Unmerchantable.

This system is used to indicate the rating of every cheese characteristic. The final grade of the cheese is the judge's estimate of the general quality on this same scale. It is not an average of the values awarded to flavor, body, texture, color and finish.

RESULTS

The 178 lots of cheese are classified into five groups:—

I. *Non-acid.* These lots were never criticised for bitterness or excessive acidity.
II. *Bitter.* Bitterness was detected in every lot.
III. *Trace-of-acid.* These lots showed only slight evidence of excessive acidity in flavor or body.
IV. *Acid.* Some acid defect was clearly evident in each cheese of this group.
V. *Sour.* These lots were very defective in flavor and body.

The quality of the cheese in each classification is indicated by the grades shown in Table 1. The average grades indicate that the best quality occurs in the non-acid group, while the worst quality appears in the sour group.

TABLE 1
Quality of cheese

CLASSIFICATION	NUMBER OF LOTS	AVERAGE GRADES		
		Flavor	Body	Final grade
Non-acid	40	3.2 ± .05	2.6 ± .07	2.9 ± .05
Bitter	37	3.4 ± .04	2.9 ± .04	3.4 ± .04
Trace-of-acid	32	3.4 ± .04	2.9 ± .06	3.4 ± .05
Acid	36	3.7 ± .05	3.2 ± .06	3.6 ± .06
Sour	33	4.0 ± .05	3.3 ± .08	4.0 ± .07

The quality of the cheese in the acid and sour groups is inferior to that of the lots in the bitter and trace-of-acid classes. The interesting fact illustrated by the data in Table 1 is the similarity of quality of the cheese in the two latter classes.

The average of the pH measurements which were made when the cheese were three days of age, is shown in Table 2. The average pH of the non-acid group, 5.2 ± .02, is slightly greater than is commonly found in Cheddar cheese of commercial quality. The means of the bitter and trace-of-acid groups are 5.05 ± .01 and 5.07 ± .02 respectively, while the acid and sour groups have mean pH values of 4.98 ± .02 and 4.93 ± .01 respectively.

TABLE 2

Acidity of cheese

CLASSIFICATION	NUMBER OF LOTS	pH ON THIRD DAY AFTER MAKING	ACID DEVELOPMENT CUTTING TO DIPPING*	ACID DEVELOPMENT CUTTING TO MILLING**
Non-acid	40	5.20 ± .02	1.79 ± .08	3.70 ± .09
Bitter	37	5.05 ± .01	2.01 ± .03	4.11 ± .08
Trace-of-acid	32	5.07 ± .02	2.34 ± .09	4.34 ± .09
Acid	36	4.98 ± .02	2.23 ± .08	4.27 ± .12
Sour	33	4.93 ± .01	2.70 ± .18	4.33 ± .13

$$\text{* Acid development} = \frac{\text{pH at cutting} - \text{pH at dipping}}{\text{Minutes from cutting to dipping}} \times 1000.$$

$$\text{** Acid development} = \frac{\text{pH at cutting} - \text{pH at milling}}{\text{Minutes from cutting to dipping}} \times 1000.$$

Approximately half of the bitter cheese at three days of age had pH values less than 5.03 which places them within the zone of acidity where acid defects may be expected (11). As a matter of fact, four-fifths of the bitter cheese were actually criticised by the judges for excessive acidity. The variability of the data is pictured graphically in figure 1. Cumulative curves have been drawn to show the percentage distribution of the lots of four classes according to the pH of the cheese, measured three days after making.

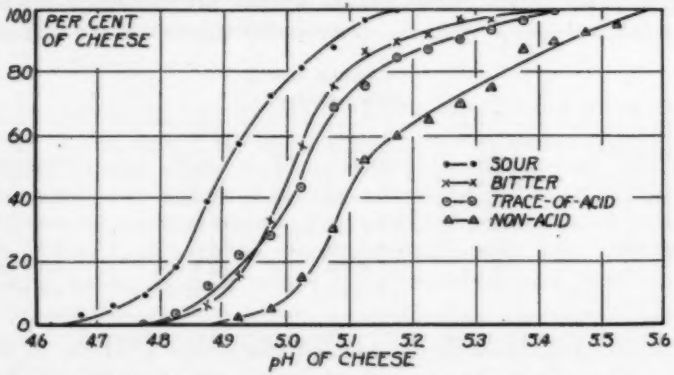

FIG. 1. Cumulative frequency curves showing the distribution of the lots of cheese in the Sour, Bitter, Trace-of-acid and Non-acid groups according to the pH measured at three days of age.

These curves clearly indicate the tendency of the cheese of the non-acid to fall in a more alkaline range than the cheese of the sour group. The curves representing bitter cheese and cheese with a trace of acid are notably similar and fall approximately midway between the two extreme classes. These curves indicate that approximately 20 per cent of the cheese samples in the non-acid class had an average pH of less than 5.05 on the third day after making, while about 85 per cent of the sour, 65 per cent of the bitter, and

60 per cent of the trace-of-acid groups had average pH values of less than 5.05 at this same age.

The rates of acid development from cutting to dipping and cutting to milling are shown in Table 2. It is usually recognized that rapid increase in acid may be responsible for defects associated with excessive acidity. The average rates of acid development in the bitter cheese from cutting to dipping and from cutting to milling, fall between the average values of the non-acid and acid-defective cheese, but approach more closely those of the latter groups.

Moisture content of curd at dipping is an indication of "firmness." Curd must be firm and without excessive acid development at dipping in order to be reasonably safe from acid defects. The average moisture contents of the cheese in each of the five groups at dipping are shown in Table 3. The differences between these means are too small to be of any great significance in distinguishing between the classes. Apparently the moisture content of bitter

TABLE 3
Moisture in cheese

CLASSIFICATION	NUMBER OF LOTS	MOISTURE IN CURD AT DIPPING	MOISTURE IN CHEESE AFTER THREE DAYS
Non-acid	40	60.3 ± .4	37.3 ± .2
Bitter	37	60.2 ± .4	37.0 ± .1
Trace-of-acid	32	59.6 ± .5	36.7 ± .2
Acid	36	59.6 ± .4	37.0 ± .2
Sour	33	59.7 ± .6	37.6 ± .3

cheese approximates that of low-acid cheese during the early stages of curd-making more closely than it does that of the high-acid type.

Cheese composition three days after making reflects the effects of mechanical treatment and acid development on moisture. The data in Table 3 show fairly low average moisture contents in the cheese of each group. Differences between groups are small, however, considering the variability indicated by the probable errors of the means. Apparently moisture content of the curd at dipping and of the cheese three days after making is not a means of distinguishing bitter cheese from either low- or high-acid cheese.

DISCUSSION

The bitter cheese and cheese with a trace of acid considered in these data have several characteristics in common. Judges found that the two groups had approximately equal quality values which made them less desirable than low-acid cheese, but superior to the high-acid products. The similar acidity attained by these two groups three days after making placed them again between the values of the extremes. The rate of acid development in the cheese of the bitter group was definitely greater than in the low-acid group, but less than any of three acid-defective groups.

It seems possible, after considering these similarities, to believe that bitterness in cheese may be associated with a slight over-acid-development. The development of high acidity in cheese may be caused by using milk in which too much acid has been permitted to develop, either on the farm or in the factory. Occasionally this over-development of acid may be caused by the use of a very active starter. Such factors also may be partly responsible for the development of too much acidity during the curd-making process. Many operators use such means to encourage high development of acidity in order to shorten the process of making. Over-development of acid during the manufacturing operations has been associated with a low pH in cheese which is three days old (11). Study of data from nearly 200 lots of American cheese showed that the pH measurements at cutting, dipping, and milling were related to the pH of the cheese at three days of age by coefficients of correlation of + .50, + .54 and + .70, respectively (12). It seems highly probable that limiting acidity development during the curd-making operations should afford an excellent means of reducing the incidence of bitter cheese.

CONCLUSION

Bitter flavor in cheese seems to be associated with the development of a slightly excessive amount of acidity.

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A STUDY OF FAT SPLITTING AND CASEIN DIGESTING BACTERIA ISOLATED FROM BUTTER

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It has long been assumed, (Hagemann), (1) that rancidity in butter is due to free butyric acid, and Richmond (2) stated that rancidity is due to the hydrolysis of fat, resulting in the presence of free volatile fatty acids. However, Schmid (3), Amthor (4), Hanus (5), Reinmann (6), Orla-Jensen (7), Sayer, Rahn, and Farrand (8), Rahn, Brown and Smith (9), and others, found that no relationship existed between rancidity in butter and the presence of free fatty acids as determined by titration methods.

Since no correlation exists between acidity and rancidity in butter, it might appear that no relation should exist between hydrolysis of fat and rancidity in butter. However, it seems highly probable that the amounts of butyric acid present in rancid butter are so small that, though they can be detected by the flavor and odor of the butter, they cannot be measured by ordinary chemical means.

Guthrie (10) stated, "A small amount of butyric acid gives the characteristic odor of rancidity. A strong solution of butyric acid does not give so characteristic a flavor of rancidity as may often be found in butter."

Holm and Greenbank, in the Fundamentals of Dairy Science by Associates of Rogers (11) say, "Milk fat, on account of its relatively high percentage of the lower fatty acids, especially butyric, readily produces a strong odor characteristic of these acids, upon slight hydrolysis."

This condition would tend to explain the negative correlation between acidity and rancidity in butter observed by workers in this field.

It has long been known that certain microorganisms can hydrolyze the fat in butter. Thus Escherich (12) stated that bacteria are able to split neutral fat to glycerol and free fatty acids, and Lafar (13) isolated two species of bacteria which produced rancidity in cream. Orla-Jensen obtained rancidity by cultivating pure and mixed cultures of organisms in butter, while Rogers (14) studied a Torula from butter which had a weak lipolytic action. Reitz (15) observed the fat splitting properties of certain bacteria, yeasts and molds. Collins (16), Hussong (17), and Hammer and Collins (18) studied fat splitting bacteria isolated from butter and the relationship of their presence in butter to its keeping quality. Since the presence of fat splitting bacteria may be of significance in the keeping qualities of butter, a detailed study has been made of 375 pure cultures of

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fat hydrolyzing bacteria isolated from butter produced and held under known and controlled conditions.

The butters used in these investigations were made under the personal supervision of Prof. E. S. Guthrie.

Turner (19) found that a nile blue sulfate medium containing fat was highly sensitive for the detection of fat splitting organisms. The toxicity of nile blue sulfate was found by him to inhibit the growth of some organisms. In this study its toxicity has been reduced by having the nile blue sulfate in solution only in the fat.

The method employed in reducing the toxicity of nile blue sulfate was developed by Prof. Georges Knaysi and has been used in his classes for several years.

The hydrolysis of milkfat by bacteria might bear little relationship to rancidity unless tributyrin were hydrolyzed producing butyric acid which is recognized as the substance necessary for the presence of rancidity in butter. The tributyrin splitting capacity of the cultures reported in this study has been determined. Anderson (20) has used a tributyrin agar for the detection of lipolytic organisms. This medium, with a few minor changes in its composition, was used by us.

It is recognized that the presence of organisms in butter capable of decomposing the casein are of importance in the deterioration of butter. Eckles (21) reported the case of a putrid butter due to proteolytic bacteria. Sayer, Rahn, and Farrand (8), and Sohgen (22), stressed the possibility of the production of strongly tasting and smelling compounds from the splitting of nitrogenous compounds in butter. Hunziker, Spitzer, Mills, and Switzer (23) suggested that protein hydrolysis may be responsible for off flavors in butter. Another investigator (24) concluded that increases in the proteolytic types of bacteria were one of the undesirable factors in butter. Sutton (25) believed that odors due to changes in the protein were a reliable indication of biological activities in butter. Spitzer and Parfitt (26) found that the score of the butter decreased as the growth of proteolytic bacteria increased. Ruehle (27) claimed that metallic flavors were due to protein decomposition products. Nelson (28) reported that protein decomposition was a very common defect in butter.

Proteolytic bacteria were studied to determine if any relationship existed between the ability of the organisms to split fat, split tributyrin, and decompose the casein of milk. Using these three properties as criteria of organisms capable of producing changes detectable by the presence of off flavors and odors in butter, this study has been made.

METHODS

Using sterile triers, portions of butter were placed into sterile test tubes which were held in a water bath at 45° C. until the butter was melted. After

mixing the contents of the tube a 1 cc. portion was removed with a pipette and placed into a sterile water blank from which triplicate agar plates were made. All materials used were warmed to 45° C. previous to use.

In pouring the plates, three agars were used. For the detection of organisms capable of hydrolyzing milk fat, the following agar was used:—

0.5 per cent beef extract
0.5 per cent yeast extract
0.5 per cent peptone
0.5 per cent glucose
1.5 per cent agar
Distilled water
pH 7.0

The agar was placed into bottles, each containing about 120 cc. Before pouring the plates, the agar was cooled to 45° C., one cc. of butter fat stained with nile blue sulfate was added and the bottle was shaken well to distribute the fat in fine globules throughout the agar. The butter fat to be stained with nile blue sulfate was melted, decanted, and placed in a large separatory funnel, and washed five or more times with hot water. A hot aqueous 5 per cent solution of nile blue sulfate was added to the butter oil in the proportion of one part nile blue sulfate to nine parts butter oil. When these materials were well mixed, and the fat had taken on a dark red color, the aqueous layer was removed and the fat washed five or more times with hot water until the water washings were free from any blue color. The fat was filtered through paper, decanted from any remaining water, tubed in 10 cc. portions and sterilized at 121° C. for 30 minutes.

The agar plates were incubated at room temperature for one week. Organisms capable of splitting the milkfat were detected by the appearance of blue or blue green granules beneath and around the colony. When the milkfat is hydrolized and the resulting fatty acids are liberated, the neutral red color of the nile blue sulfate in the presence of these fatty acids is changed to a blue or blue green. Well isolated fat splitting colonies were picked from these agar plates into a medium of the following composition:—

0.5 per cent beef extract
0.5 per cent yeast extract
0.5 per cent peptone
0.5 per cent glucose
Distilled water
pH 7.0

This broth was used throughout this study as a stock medium for preparing the cultures for various tests.

For the detection of organisms capable of hydrolyzing tributyrin, a medium of the same composition was used for plating, except that glucose

was omitted. Previous to pouring the plates, one cc. of sterile tributyrin (Eastman-Kodak Co. C. P.) was added to the agar, which was well shaken to distribute the oil evenly throughout the agar. The plates were incubated at room temperature for one week. The organisms capable of splitting tributyrin were detected by the presence of a clear zone around the colony distinguished from the opaque emulsified tributyrin in the rest of the agar. Upon the hydrolysis of the insoluble tributyrin, butyric acid and glycerol are formed, these compounds being soluble in the agar. The tributyrin splitters were picked into a broth of the same composition as that used for the isolation of the fat hydrolyzing bacteria.

For the detection of organisms capable of decomposing the casein in milk, an agar medium of the same composition as that used for the fat splitters was employed, the fat being omitted, and five cc. portions of sterile milk added to each bottle of agar just before pouring the plates. These agar plates were held for one week at room temperature. The milk plates were opaque and cloudy in appearance and proteolytic organisms were detected by the presence of a clear zone around the colony not becoming turbid when the agar was acidified by flooding the surface of the agar with tannic acid. Well isolated colonies were placed directly into litmus milk which was held for two weeks in order to confirm their action on casein.

To determine the relationship between the ability of the organisms to split fat, split tributyrin, and decompose the casein in milk, each culture isolated was streaked upon all of the three agars used in this study.

Motility tests were determined from hanging drop preparations of actively growing cultures. From agar slope cultures, the gram reaction of the organisms was determined using Burke's (29) modification of the gram staining technique. These microscopic examinations also furnished additional confirmation of the purity of the cultures being studied.

Acid and gas production from certain carbohydrate materials was determined by inoculation into standard nutrient broth, to which had been added 0.5 per cent of the carbohydrate, contained in Durham tubes. To aid in classifying the aerobic spore-producing rods, their activity on starch was determined by streaking upon a starch agar consisting of nutrient agar to which had been added 0.2 per cent water soluble starch. Each culture was tested for its ability to liquefy gelatin. Cultures grown in peptone broth were tested with Nessler's reagent for ammonia production, and with Ehrlich's reagent for the production of indol. Ability to reduce nitrates was determined by inoculating nitrate broth contained in Durham tubes. The presence of nitrites was detected by using Trommsdorf's reagent and further reduction by the presence of gas in the inverted vial.

Precautions were taken to be certain that all the test media were inoculated with vigorously growing cultures. In all the tests for the activity of these organisms on various substances, a two weeks holding period at room temperature was employed.

Temperature limits of growth of all the organisms studied were determined by inoculation into a medium of the following composition:—

0.5 per cent beef extract
0.5 per cent yeast extract
0.5 per cent peptone
0.1 per cent glucose
Distilled water
pH 7.0

Immediately after inoculation the temperature of the broth was adjusted to the conditions under which it was to be incubated for two weeks.

Representatives of each group studied were tested for the ability to withstand a temperature of 62.8° C. (145° F.) for 30 minutes. One cc. of a 24-hour broth culture was placed into the bottom of a sterile test tube, precautions being taken not to let any of the broth culture touch the side of the test tube. Nine cc. of sterile silk were carefully added with a pipette in such a manner as to mix the contents of the tube. The cotton plugs were replaced with sterile rubber stoppers to avoid the cooler temperatures which would prevail at the surface of the liquid during the heating period if evaporation were allowed to take place. The tubes were placed in a constant temperature water bath which had been held at 62.8° C. (145° F.) for four hours previous to testing the cultures. The temperature did not vary more than $\pm$ 0.1° C. during the holding period. After being subjected to this temperature for 30 minutes, the tubes were immediately cooled in ice water and the rubber stoppers replaced with cotton plugs. In all cases, growth in the pasteurized milk was detected by streaking agar slopes from the milk at the end of one and two weeks of incubation at room temperature. Casein digesters which survive the heating process could also be detected by their action upon the milk. All organisms surviving the heat treatment were retested.

EXPERIMENTAL

The groupings which have been made in this study are based upon Bergey's (30) classification. The activity of these organisms on milkfat and tributyrin has been included.

Of the organisms isolated, 188 were found to be gram negative rods (Tables 1 and 1a), of which eleven were members of the Escherichia-Aerobacter group. Forty cultures which resembled *Pseudomonas aeruginosa* in physiological properties have been studied. Thirty of these cultures produced a blue-green pigment soluble in water, turning to a dark brown with age, and becoming red in the presence of acid. In testing for indol production a red color was produced but it was insoluble in chloroform, in this way differing from Bergey's description of this species. The other ten cultures were identical in all respects with the above type except that

TABLE 1

Some physiological properties of certain gram negative rods isolated from butter

NAME OF ORGANISMS	NUMBER OF CULTURES	MOTILITY	ACTION ON LITMUS MILK					LIQUEFACTION OF GELATIN	HYDROLYSIS OF MILKFAT	HYDROLYSIS OF TRIBUTYRIN	ACTION ON SUGARS			REDUCTION OF NITRATES	AMMONIA FROM PEPTONE	INDOL FROM PEPTONE	TEMPERATURE LIMITS OF GROWTH							
			Digestion	Curd	Acid	Alkaline	Reduction of litmus				Glucose	Lactose	Sucrose				5° C.	10° C.	15° C.	Room	37° C.	40° C.	45° C.	50° C.
Pseudomonas aeruginosa	30	+	+	–	–	–	–	+	+	+	–	–	–	⊕	+	–	–	+	+	+	+	+	+	–
Pseudomonas aeruginosa, variant	10	+	+	–	–	–	–	+	+	+	–	–	–	⊕	+	–	–	±	+	+	+	+	+	–
Achromobacter pellucidum	37	+	+	–	–	+	–	+	+	+	–	–	–	–	+	–	±	+	+	+	+	±	±	–
Achromobacter pellucidum, variant	16	+	+	–	–	+	–	+	+	+	–	–	–	+	+	–	±	+	+	+	+	±	±	–
Achromobacter aromafaciens	18	+	sl.*	+	–	–	+	+	+	+	–	–	–	–	+	–	+	+	+	+	–	–	–	–
Achromobacter Sp.	3	+	+	+	–	–	–	+	+	+	+	–	–	–	+	–	+	+	+	+	+	–	–	–
Achromobacter Sp.	4	±	–	–	–	sl.	–	–	±	+	+	–	–	–	±	–	±	+	+	+	+	–	–	–
Alcaligenes bookerii (I)	23	+	+	+	–	–	+	+	+	+	–	–	–	–	+	–	+	+	+	+	+	–	–	–
Alcaligenes bookerii (II)	7	+	+	+	–	–	–	+	+	+	–	–	–	–	+	–	–	+	+	+	+	±	–	–
Alcaligenes bookerii (III)	10	+	+	+	–	–	–	+	+	+	–	–	–	+	+	–	–	+	+	+	+	±	–	–
Alcaligenes fecalis	6	+	–	–	–	±	±	–	±	+	–	–	–	–	+	–	±	+	+	+	+	–	–	–
Alcaligenes fecalis, variant	6	–	–	–	–	+	–	–	+	+	–	–	–	–	+	–	±	±	+	+	±	±	±	–
Flavobacterium synxanthum	4	+	+	–	–	–	–	+	–	+	+	–	+	–	+	–	+	+	+	+	+	+	–	–
Flavobacterium Sp.	3	+	–	–	–	–	+	+	–	+	+	–	+	+	+	–	+	+	+	+	+	–	–	–

* sl. = slight.

TABLE 1a

Some physiological properties of members of the Escherichia-Aerobacter group isolated from butter

NAME OF ORGANISMS	NUMBER OF CULTURES	MOTILITY	ACTION ON LITMUS MILK					LIQUEFACTION OF GELATIN	HYDROLYSIS OF MILKFAT	HYDROLYSIS OF TRIBUTYRIN	ACTION ON CARBOHYDRATES					VOGES-PROSKAUER TEST	METHYL RED TEST	INDOL FROM PEPTONE	REDUCTION OF NITRATES	AMMONIA FROM PEPTONE	TEMPERATURE LIMITS OF GROWTH				
			Digestion	Curd	Acid	Alkaline	Reduction of litmus				Glucose	Lactose	Sucrose	Salicin	Dulcitol						5° C.	10° C.	40° C.	45° C.	50° C.
Aerobacter cloacae	8	+	−	+	+	−	+	+	−	+	⊕	⊕	⊕	⊕	−	+	−	−	⊕	+	−	+	+	+	−
Intermediate Sp. ...	1	+	−	+	+	−	+	−	+	+	⊕	⊕	⊕	⊕	−	−	−	−	⊕	+	+	+	+	−	−
Intermediate Sp. ...	1	+	−	+	+	−	+	+	−	+	⊕	⊕	⊕	⊕	−	−	−	−	+	+	−	+	+	+	−
Escherichia Sp.	1	−	−	+	+	−	+	−	−	+	⊕	⊕	⊕	⊕	⊕	−	+	−	⊕	+	+	+	+	+	−

⊕ = acid and gas.

they produced a yellow-green pigment slightly soluble in water, the color of which did not change with age or in the presence of acid. Under the same conditions no blue pigment was produced by these cultures. This group was assumed to be a variant of *Pseudomonas aeruginosa*. All 40 cultures hydrolyzed milkfat and tributyrin and digested the casein of milk.

Another large group studied consisted of 53 cultures which resembled *Achromobacter pellucidum*. Thirty-seven of the cultures were true to the described type but the remaining 16 cultures were able to reduce nitrates. This entire group was active on milkfat, tributyrin, and the casein of milk.

Eighteen cultures resembling Bergey's description of *Achromobacter aromafaciens* were studied. The cultures described in this work were motile, whereas Bergey reports the type species as non-motile. These cultures were active on milkfat and tributyrin, and exhibited a slight action upon the casein of milk.

Three cultures slightly resembled *Achromobacter lipolyticum* and were active on milkfat and tributyrin and peptonized the casein of milk. These cultures were unable to ferment maltose, sucrose, glycerol and mannitol, thereby differing from Bergey's description of the species. *Achromobacter* sp. consisted of four cultures, two of which were motile, produced ammonia, and acted on fat; all acted on tributyrin and none digested casein.

Another group of 40 cultures resembling Bergey's description of *Alcaligenes bookeri* really consisted of three separate groups. Twenty-three of the cultures definitely reduced the litmus in litmus milk which the remaining 17 cultures did not do. Thirty of the 40 cultures did not reduce nitrates. Bergey's statement regarding the reduction of nitrates by *Alcaligenes bookerii* is vague and indefinite. Some similarity can be noticed between *Alcaligenes bookerii* and *Achromobacter aromafaciens*, the major difference being in the extent to which the casein of milk was digested, and the temperature limits of growth. *Achromobacter aromafaciens* is exceptional because of its inability to grow at 37° C. All of these cultures were active on milkfat and tributyrin, and peptonized the casein of milk.

The *Alcaligenes fecalis* group consisted of 12 cultures which agreed closely with Bergey's description. Six of the cultures differed from the type species in being nonmotile and are referred to as variants. Ten of the cultures isolated were able to split milkfat, all 12 split tributyrin, and none were active on the casein of milk.

Seven of the gram negative rods studied produced a yellow pigment. The first group, *Flavobacterium synxanthum*, consisting of four cultures, was active on tributyrin and the casein of milk, but was unable to split milkfat. *Flavobacterium* sp. split tributyrin and did not attack the milkfat or the casein of milk.

Of the eleven cultures of gas producing gram negative rods belonging to the Escherichia-Aerobacter group, none was able to attack the casein

of milk. Eight cultures, identified as *Aerobacter cloacae*, split tributyrin but were unable to attack the milkfat. The *Escherichia* sp. and the two intermediates were active on tributyrin, only one of which was able to split milkfat.

Of the 188 cultures of gram negative rods here reported, only the *Flavobacterium synxanthum* cultures were able to survive pasteurization at 62.8° C. (145° F.) for 30 minutes. Certain cultures of blue pigment producing *Pseudomonas aeruginosa* showed on holding a slow peptonization of the pasteurized milk, but when streaks were made upon agar slants from the milk, no growth occurred. This observation indicated the presence of proteolytic enzymes which were not destroyed by the heat treatment. The enzyme-protecting effect of soluble proteins has also been observed by Virtanen and Tarnanen (31).

Eighty-four per cent (158) of the 188 cultures of gram negative rods studied digested milk, 89 per cent (167) split fat, and 100 per cent (188) hydrolyzed tributyrin. Of the 158 cultures which proteolized milk, 97 per cent also split fat. It might be significant to mention that though all of these 158 cultures were able to split tributyrin, 47 per cent of them were isolated on the basis of their ability to split fat, 30 per cent because of their power to digest casein, and the remaining 23 per cent for their ability to hydrolyze tributyrin. Another fact of importance is that these organisms, all of which were isolated from sweet cream, unsalted butter, were present in many millions per gram. Such large numbers are recognized as significant (32), (33).

A study has been made of 214 cultures of cocci, of which 151 were gram positive micrococci, 40 gram negative micrococci, and 23 gram positive sarcina. Because of the incomplete descriptions of the characteristics of the organisms recorded in Bergey's Manual, it is difficult to be certain of the names under which these organisms should be reported. It has been felt better to use names in the instances where the number of cultures having like properties was large. When possible, previously used names have been employed. In five instances, the reactions given by the groups of cultures studied were so outstanding, yet so different from previously recorded descriptions, that new names are believed justified.

Some of the physiological properties of 40 cultures of gram negative cocci occurring singly, in pairs, or irregular masses, are recorded in Table 2. The outstanding characteristic of all of these cultures is their ability to hydrolize fats. Since the findings recorded in the literature did not describe these organisms, it is suggested that the type species of the group be called *Micrococcus lipolyticus* n. sp. All of the cultures attacked both fat and tributyrin, but were unable to digest casein. The source of these organisms was sweet cream, unsalted butter. None was able to survive pasteurization at 62.8° C. (145° F.) for 30 minutes. Since these organisms were found in butter in millions per gram, they are probably of significance in its spoilage.

TABLE 2

Some physiological properties of Micrococcus lipolyticus and certain related species

NAME OF ORGANISMS	NUMBER OF CULTURES	ACTION ON LITMUS MILK					LIQUEFACTION OF GELATIN	HYDROLYSIS OF MILKFAT	HYDROLYSIS OF TRIBUTYRIN	ACTION ON SUGARS			REDUCTION OF NITRATES	AMMONIA FROM PEPTONE	INDOL FROM PEPTONE	TEMPERATURE LIMITS OF GROWTH						
		Digestion	Curd	Acid	Alkaline	Reduction of litmus				Glucose	Lactose	Sucrose				5° C.	10° C.	Room	37° C.	40° C.	45° C.	50° C.
Micrococcus lipolyticus N. Sp.	22	–	–	–	+	sl.	–	+	+	–	–	–	–	+	–	+	+	+	+	–	–	–
Micrococcus lipolyticus, variant	12	–	–	–	sl.	–	–	+	+	–	–	–	–	w+	–	+	+	+	+	+	–	–
Micrococcus lipolyticus, variant	3	–	–	–	+	–	–	+	+	–	–	–	–	+	–	–	+	+	+	+	+	±
F 77	1	–	–	–	–	–	–	+	+	–	–	–	–	+	–	+	+	+	+	+	+	–
F 215, 216	2	–	–	–	–	–	–	+	+	+	–	–	–	+	–	+	+	+	–	–	–	–

sl. = slight.
w = weak.

TABLE 3

Some reactions given by Sarcina caseolytica and certain related species

NAME OF ORGANISMS	NUMBER OF CULTURES	ACTION ON LITMUS MILK					LIQUEFACTION OF GELATIN	HYDROLYSIS OF MILKFAT	HYDROLYSIS OF TRIBUTYRIN	ACTION ON SUGARS			REDUCTION OF NITRATES	AMMONIA FROM PEPTONE	INDOL FROM PEPTONE	TEMPERATURE LIMITS OF GROWTH					PIGMENTATION
		Digestion	Curd	Acid	Alkaline	Reduction of litmus				Glucose	Lactose	Sucrose				5° C.	10° C.	40° C.	45° C.	50° C.	
Sarcina caseolytica N. Sp. ...	14	+	+	−	−	+	+	−	−	−	−	+	+	+	−	−	+	+	+	−	white
Sarcina caseolytica, variant	4	+	+	−	−	+	+	−	−	−	−	−	+	+	−	−	+	+	+	−	white
P 28	1	+	+	−	−	+	+	−	−	−	−	−	−	−	−	−	+	+	+	−	white
P 95	1	+	+	−	−	+	+	−	+	+	−	−	+	+	−	−	+	+	+	−	white
T 108, 109	2	−	−	+	−	−	−	−	+	+	+	+	+	−	−	±	+	+	−	−	white
C 27	1	−	−	−	−	−	+	+	+	+	−	+	−	+	−	−	+	+	+	−	white

Twenty-three of the isolations made were gram positive organisms belonging to the Sarcina genus (Table 3). Fourteen of the cultures were similar in all the physiological properties tested, and were very active on the casein of milk. The only milk digesting sarcina listed in Bergey's Manual is *Sarcina aurantiaca* which differed from the cultures here reported in pigment production, nitrate reduction, indol production and sugar fermentations. Since these organisms occurred in butter in large enough numbers to be of significance in its keeping quality, and since their most outstanding physiological property was their active digestion of casein in milk, it was decided to suggest for them the name *Sarcina caseolytica* n. sp. Four other cultures varied from the type species only in their inability to ferment sucrose. None of these 18 cultures were able to hydrolize either fat or tributyrin. Five other cultures of sarcina, the physiological reactions of which are reported in Table 3, varied from the type species in other respects. None of these cultures were able to survive pasteurization at 62.8° C. (145° F.) for 30 minutes. The source of these organisms was salted and unsalted, raw cream butter. Difficulty was experienced in keeping these cultures alive.

Thirty-eight of the gram positive micrococcus cultures studied (Table 4) were morphologically different from any of the species previously described in the literature. These cultures when aged were definitely micrococci, but in their early stages of growth were distinctly rod-like to such an extent that one might mistake them for short oval rods. The physiological properties of these organisms were very similar to those of *Micrococcus lipolyticus* but due to the difference in the gram reaction and their variable morphology, the name *Micrococcus intermedius* n. sp. is offered. Twenty-six of the cultures were alike and produced a moist, light yellow growth on agar slopes, caused litmus milk to become alkaline, failed to reduce nitrates, and were active in the hydrolysis of milkfat and tributyrin. Six other cultures in this group varied from the type species only in their action upon milk, or nitrates, or milkfat. Six miscellaneous cultures were morphologically related to the type species but were physiologically more variable.

Fifty-three medium-sized gram positive micrococci, occurring in irregular clumps, have been studied. Twenty of the cultures were alike and resembled *Micrococcus flavus* except that the cultures here reported reduced nitrates. Five other cultures in this group were similar to *Micrococcus freudenreichii* except that these cultures produced a light yellow pigment. The variants of both *Micrococcus flavus* and *Micrococcus freudenreichii* were active in the hydrolysis of milkfat and tributyrin and in the digestion of casein, which may be indicative of their importance in the spoilage of butter. The remaining 28 miscellaneous cultures varied in other respects. These results are summarized in Table 5.

TABLE 4

Some of the reactions given by Micrococcus intermedius and some related species

NAME OF ORGANISMS	NUMBER OF CULTURES	PIGMENTATION	ACTION ON LITMUS MILK					LIQUEFACTION OF GELATIN	HYDROLYSIS OF MILKFAT	HYDROLYSIS OF TRIBUTYRIN	ACTION ON SUGARS			REDUCTION OF NITRATES	AMMONIA FROM PEPTONE	INDOL FROM PEPTONE	TEMPERATURE LIMITS OF GROWTH						
			Digestion	Curd	Acid	Alkaline	Reduction of litmus				Glucose	Lactose	Sucrose				5° C.	10° C.	15° C.	37° C.	40° C.	45° C.	50° C.
Micrococcus intermedius N. Sp.	26	light yellow	–	–	–	+	–	–	+	+	–	–	–	–	+	–	±	+	+	+	±	±	–
Micrococcus intermedius, variant	3	light yellow	–	–	–	–	–	–	+	+	–	–	–	–	+	–	–	+	+	+	+	–	–
Micrococcus intermedius, variant	1	light yellow	–	–	–	+	–	–	–	+	–	–	–	–	+	–	–	+	+	+	–	–	–
Micrococcus intermedius, variant	1	light yellow	–	–	–	–	–	–	+	+	–	–	–	+	+	–	–	+	+	+	+	–	–
Micrococcus intermedius, variant	1	yellow	–	–	–	–	–	–	+	+	–	–	–	+	+	–	–	+	+	+	+	+	–
T 227, 228	2	deep orange	–	–	–	+	–	–	–	+	–	–	–	–	+	–	–	+	+	+	–	–	–
T 269	1	light yellow	–	–	–	–	–	–	+	+	+	–	–	–	+	–	–	+	+	+	+	–	–
T 226	1	light yellow	+	–	–	–	+	+	–	+	+	–	+	–	+	–	+	+	+	+	+	+	–
F 248	1	pale yellow	–	–	–	+	–	–	+	+	+	–	+	–	+	–	–	+	+	+	+	+	–
P 220	1	pink	–	–	–	+	–	–	+	+	–	–	–	–	–	–	–	–	+	+	–	–	–

TABLE 5

The physiological reactions of some medium-sized gram positive Micrococci isolated from butter

NAME OF ORGANISM	NUMBER OF CULTURES	PIGMENTATION	ACTION ON LITMUS MILK					LIQUEFACTION OF GELATIN	HYDROLYSIS OF MILKFAT	HYDROLYSIS OF TRIBUTYRIN	ACTION ON SUGARS			REDUCTION OF NITRATES	AMMONIA FROM PEPTONE	INDOL FROM PEPTONE	TEMPERATURE LIMITS OF GROWTH						
			Digestion	Curd	Acid	Alkaline	Reduction of litmus				Glucose	Lactose	Sucrose				5° C.	10° C.	15° C.	37° C.	40° C.	45° C.	50° C.
Micrococcus flavus variant	20	orange	+	+	+	−	+	+	+	+	+	+	+	+	+	−	−	+	+	+	+	+	−
Micrococcus freudenreichii, variant	5	light yellow	+	±	+	−	+	+	+	+	+	+	+	−	+	−	±	+	+	+	+	±	−
P 22	1	orange	−	+	+	−	+	−	+	+	+	+	+	+	+	−	−	+	+	+	+	+	−
F 233	1	light yellow	−	+	+	−	+	+	+	+	+	+	+	−	+	−	+	+	+	+	+	−	−
F 79, 80	2	white	−	−	+	−	+	+	+	+	+	+	+	−	+	−	−	+	+	+	+	+	−
F 235, 236	2	white	−	−	+	−	−	−	+	+	+	+	+	−	+	−	−	+	+	+	+	+	−
P 231, 232	2	yellow	+	+	+	−	+	+	−	+	+	+	+	+	+	−	−	+	+	+	+	+	−
T 289, 290	2	yellow	−	−	+	−	−	−	−	+	+	+	+	+	−	−	+	+	+	+	−	−	−
F 210	1	white	−	−	−	−	−	+	+	+	+	−	+	−	+	−	−	+	+	+	+	−	−
F 204	1	white	−	−	−	−	−	−	+	+	+	−	+	+	−	−	−	−	+	+	+	−	−
T 230	1	white	−	−	−	−	−	−	+	+	+	−	+	−	+	−	+	+	+	+	+	+	−
T 279	1	yellow	−	−	−	+	+	−	+	+	+	−	+	−	−	−	−	+	+	+	+	−	−
T 221, 222, 257, 261	4	lemon yellow	−	−	+	−	+	−	−	+	+	+	−	+	−	−	+	+	+	+	±	−	−
T 249, 250	2	lemon yellow	−	−	+	−	+	−	+	+	+	+	−	+	−	−	−	+	+	+	+	+	+
P 221, 270, 271	3	lemon yellow	+	+	+	−	+	+	−	+	+	+	−	+	−	−	−	+	+	+	+	+	+
T 280	1	lemon yellow	+	+	+	−	+	+	+	+	+	+	−	+	−	−	−	+	+	+	+	−	−
P 24, 25	2	white	−	+	+	−	+	+	+	+	+	+	−	−	+	−	−	−	+	+	+	+	−
T 29, 30	2	orange	+	+	+	−	+	+	+	+	+	+	−	+	−	−	−	±	+	+	+	+	±

TABLE 6

The physiological reactions of some large gram positive Micrococci isolated from butter

NAME OF ORGANISM	NUMBER OF CULTURES	PIGMENTATION	ACTION ON LITMUS MILK					LIQUEFACTION OF GELATIN	HYDROLYSIS OF MILKFAT	HYDROLYSIS OF TRIBUTYRIN	ACTION ON SUGARS			REDUCTION OF NITRATES	AMMONIA FROM PEPTONE	INDOL FROM PEPTONE	TEMPERATURE LIMITS OF GROWTH						
			Digestion	Curd	Acid	Alkaline	Reduction of litmus				Glucose	Lactose	Sucrose				5° C.	10° C.	15° C.	37° C.	40° C.	45° C.	50° C.
Micrococcus caseolyticus, variant	8	yellow	+	+	+	−	+	+	−	+	+	+	+	+	+	−	+	+	+	+	+	+	−
Micrococcus magnus N. Sp.	21	white	−	±	+	−	+	−	−	+	+	+	+	−	+	−	±	±	+	+	±	±	+
Micrococcus tributyrus N. Sp.	5	light yellow	−	−	−	−	−	−	−	+	−	−	−	+	+	−	±	+	+	+	+	±	±
F 58	1	white	−	+	+	−	+	−	+	+	+	+	+	−	+	−	−	+	+	+	+	+	−
T 235	1	white	−	−	−	−	−	−	+	+	+	+	+	+	+	−	+	+	+	+	+	−	−
T 99	1	white	−	−	−	−	−	−	+	+	+	+	−	−	+	−	−	+	+	+	+	+	−
T 48, 61, 62	3	white	−	±	−	−	+	−	+	+	+	−	+	+	+	−	±	+	+	+	+	±	−
T 72, 85	2	white	+	+	−	−	+	+	−	+	+	−	+	+	+	−	−	+	+	+	+	−	−
T 53	1	slight yellow	−	−	−	+	−	−	+	+	+	−	+	+	+	−	+	+	+	+	+	+	−
T 54	1	white	−	−	−	−	+	−	+	+	+	−	+	−	+	−	−	+	+	+	+	+	−
T 74	1	slight yellow	−	−	−	+	+	+	−	+	+	−	+	+	+	−	−	+	+	+	−	−	−
P 105	1	yellow	+	−	−	−	+	+	−	+	+	−	+	−	+	−	+	+	+	+	+	+	−
T 272	1	pink	−	−	−	−	−	−	−	+	+	−	+	−	−	−	+	+	+	+	−	−	−
T 111	1	white	+	+	+	−	+	+	−	+	+	+	+	−	+	−	−	+	+	+	+	+	−
T 79, 80	2	slight yellow	−	−	−	−	+	−	−	+	+	−	−	+	+	−	−	±	+	+	+	+	−
T 47	1	white	+	+	+	−	+	+	+	+	+	+	−	+	+	−	+	+	+	+	+	+	−
P 103	1	yellow tan	+	−	−	−	+	+	−	+	+	−	−	−	+	−	+	+	+	+	+	−	−
T 101, 103	2	white	−	−	−	−	−	−	−	+	+	−	−	+	+	−	+	+	+	+	+	−	−
T 93, 102	2	orange	−	−	−	−	−	−	−	+	+	−	−	+	+	−	+	+	+	+	+	−	−
T 57, 58	2	white	−	−	−	−	−	−	+	+	+	−	−	−	+	−	−	−	+	+	+	−	−
T 295, 296	2	yellow	−	−	−	−	+	−	−	+	−	−	−	+	+	−	+	+	+	+	−	−	−

The remaining gram positive micrococci (Table 6), consisting of 60 cultures, were very large spherical forms occurring singly or in irregular masses. One of the noticeable properties of these cultures was the difficulty experienced in keeping them alive. A gram stained preparation from an agar slope would invariably show many gram negative cocci in each microscopic field, probably indicating that these organisms were dead. Eight of these cultures resembled *Micrococcus caseolyticus* except that they produced a yellow pigment. Twenty-one other cultures in this group produced acid and slight reduction in litmus milk, and hydrolyzed tributyrin. Since the reactions of these organisms were not found in any of the recorded descriptions, and since no outstanding physiological property was noted, and because their morphology was distinct, the name *Micrococcus magnus* n. sp. has been used. Five other cultures caused no change in litmus milk, and no action on the milkfat or the carbohydrates tested. Since these cultures, although few in number, appeared to be both distinct and different from any other description reported, a new name seemed necessary. Because these cultures were isolated from butter, and also their action in hydrolyzing tributyrin may indicate their importance in the spoilage of butter, the name *Micrococcus tributyrus* n. sp. is suggested. The reactions of the remaining 26 miscellaneous cultures are shown in Table 6. No further classification has been attempted.

None of the 214 cultures of cocci studied were able to survive pasteurization at 62.8° C. (145° F.) for 30 minutes. The predominant source from which these cocci were isolated was sweet cream, salted and unsalted butter, although a few isolations were made from sour cream butter. With the exception of the gram negative micrococci and sarcina groups, of which previous mention has been made, the cocci here reported did not occur in sufficiently large numbers in butter to be of great importance in its spoilage.

Ten cultures of gram positive nonsporeproducing rods (Table 7) have been studied. Six of these cultures (Group A) are short, thin, slender rods occurring singly or in short chains. On ordinary agar media they produced an abundant, moist, white growth. Litmus milk was reduced and peptonized, both milkfat and tributyrin were hydrolyzed, and none of the carbohydrates tested was fermented. Growth occurred at 5° C. but not at 37° C. These organisms were present in butter in large enough numbers to be of possible significance in its spoilage, but since they were observed in only one sample of butter, their importance might be questioned. No name is suggested. Four other miscellaneous cultures of gram positive nonsporeproducing rods were studied (see Table 7).

Of 74 gram positive, aerobic, sporeproducing rods studied (Table 8), 34 cultures were identified as *Bacillus mesentericus*. The typical organism of this group is very active in the liquefaction of gelatin and the digestion of milk, ferments glucose and sucrose, and does not hydrolyze starch or reduce

TABLE 7

The reactions of some gram positive non-spore producing rods isolated from butter

NAME OF ORGANISMS	NUMBER OF CULTURES	MOTILITY	ACTION ON LITMUS MILK					LIQUEFACTION OF GELATIN	HYDROLYSIS OF MILKFAT	HYDROLYSIS OF TRIBUTYRIN	ACTION ON SUGARS			REDUCTION OF NITRATES	AMMONIA FROM PEPTONE	INDOL FROM PEPTONE	TEMPERATURE LIMITS OF GROWTH						
			Digestion	Curd	Acid	Alkaline	Reduction of litmus				Glucose	Lactose	Sucrose				5° C.	10° C.	Room	37° C.	40° C.	45° C.	50° C.
Group A	6	+	+	+	−	−	+	+	+	+	−	−	−	−	+	−	+	+	+	−	−	−	−
P 4	1	−	+	+	−	−	+	+	−	−	−	−	+	+	+	−	−	+	+	+	+	+	−
P 273	1	−	−	+	+	−	+	−	−	+	+	+	+	−	+	−	+	+	+	+	+	−	−
A 53	1	−	−	−	−	−	−	−	+	+	+	+	−	−	+	−	−	+	+	+	−	−	−
P 108	1	−	−	−	−	+	−	−	+	+	−	−	−	−	+	−	+	+	+	+	−	−	−

TABLE 8

Some physiological properties of gram positive spore producing rods isolated from butter

NAME OF ORGANISMS	NUMBER OF CULTURES	MOTILITY	ACTION ON LITMUS MILK					LIQUEFACTION OF GELATIN	HYDROLYSIS OF MILKFAT	HYDROLYSIS OF TRIBUTYRIN	ACTION ON CARBOHYDRATES				REDUCTION OF NITRATES	AMMONIA FROM PEPTONE	INDOL FROM PEPTONE	TEMPERATURE LIMITS OF GROWTH						
			Digestion	Curd	Acid	Alkaline	Reduction of litmus				Glucose	Lactose	Sucrose	Starch				5° C.	10° C.	15° C.	37° C.	40° C.	45° C.	50° C.
Bacillus mesentericus	34	+	+	−	−	−	+	+	+	+	+	−	+	−	−	+	−	−	+	+	+	+	+	±
Bacillus Sp.	28	+	+	−	−	+	+	+	+	+	−	−	−	−	+	+	−	−	±	+	+	+	±	−
Bacillus parvus	1	+	+	−	−	+	−	+	+	+	−	−	−	+	−	+	−	−	−	+	+	+	+	+
Bacillus agri	1	+	+	−	−	−	+	+	+	+	−	−	−	−	−	+	−	+	+	+	+	+	+	−
Bacillus laterosporous	2	+	+	−	−	−	+	+	+	+	+	−	−	−	−	+	−	−	+	+	+	+	+	+
Bacillus simplex	1	+	+	−	−	+	+	+	+	+	+	−	−	−	+	+	−	−	−	+	+	+	+	−
Bacillus mycoides	1	+	w+	−	−	−	+	+	+	+	+	−	+	+	+	+	−	−	−	+	+	+	+	+
Bacillus cereus	1	+	+	−	−	−	+	+	+	+	+	−	+	+	+	+	−	−	−	+	+	+	+	+
Bacillus ruminatus	1	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	−	−	+	+	+	+	+	−
Bacillus peptogenes	1	−	+	+	+	−	+	+	+	+	+	+	+	+	−	+	−	−	+	+	+	+	+	−
Bacillus subtilis	1	+	+	+	−	−	+	+	+	+	+	−	+	+	+	+	−	−	−	+	+	+	+	+
Bacillus Sp.	1	−	+	−	−	−	+	+	+	+	−	−	−	+	+	+	−	−	+	+	+	+	+	−
Bacillus Sp.	1	+	−	−	+	−	+	−	−	+	+	+	−	−	−	+	−	+	+	+	+	−	−	−

w = weak.

nitrates. Previous studies have not reported the action of these organisms on milkfat or tributyrin. These 34 cultures were very active in the hydrolysis of these fats. Twenty-eight of the cultures studied resembled the morphological description reported for *Bacillus centrosporous* but differed materially in cultural characteristics and physiological properties from any description recorded by Bergey. These organisms were also active in the digestion of casein and the splitting of milkfat and tributyrin. Ten other cultures were identified as *Bacillus parvus, Bacillus agri, Bacillus laterosporous, Bacillus simplex, Bacillus mycoides, Bacillus cereus, Bacillus ruminatus, Bacillus peptogenes,* and *Bacillus subtilis.* Two unidentified cultures were found.

With one exception, these 74 cultures were active in splitting milkfat and tributyrin and digesting casein. Although the presence of large numbers of these organisms in butter would obviously be harmful, the numbers found in the samples tested in this study were never large enough to indicate significant growth of these organisms in butter. Since they were never present in numbers greater than a few hundred to a few thousand per gram of butter, they would seem to be of no practical importance in its deterioration. It is believed, however, that large numbers of such organisms present in the cream previous to the making of the butter would affect its keeping quality.

DISCUSSION

In this investigation, agar containing fat, tributyrin, or milk has been used in determining both total and differential counts of micro-organisms present in samples of butter made and held under known and controlled conditions. From these butters, 486 pure cultures have been isolated on the basis of their ability to hydrolyze fat, or split tributyrin, or digest casein. Thirty-eight per cent (183 cultures) were isolated from milkfat agar, 36 per cent (174 cultures) from tributyrin agar, and 26 per cent (129 cultures) from milk agar. A study of the cultures showed 96 per cent (467 cultures) were tributyrin positive, 77 per cent (375 cultures) were fat positive, and 63 per cent (306 cultures) were proteolytic on milk.

Of the 486 cultures reported in this study, 188 were gram negative rods, 40 were gram negative micrococci, 23 were gram positive sarcina, 151 were gram positive micrococci, 10 were gram positive nonsporeproducing rods, and 74 were gram positive aerobic sporeproducing rods.

Those present in large enough numbers to be of importance in the spoilage of butter were gram negative rods, gram negative micrococci, gram positive sarcina and gram positive nonsporeproducing rods. Of these groups which occurred in large numbers, only the gram negative rods were regularly present in all of the butters tested. One hundred fifty-eight or 84 per cent of the cultures of gram negative rods were able to digest casein in milk agar. Since 97 per cent (154 cultures) of these organisms hydrolyzed

fat, and all of them split tributyrin, it would seem that the presence of casein-digesting gram negative rods in butter or cream might well be used as an indication of the probable spoilage of butter (34). Small numbers of these proteolytic gram negative rods found in fresh lightly salted, sweet cream butter, held at temperatures which would permit the growth of these organisms, was invariably followed by large numbers of the organisms and a poor quality butter.

Although either tributyrin or milkfat agar might appear to be a more sensitive criterion in determining the spoilage of butter by micro-organisms, the toxicity of the tributyrin, the slight toxicity of the nile blue sulfate, and the difficulties involved in the preparation and use of these media would probably preclude their general use.

The relation of these bacteria to the keeping qualities of various types of butters made and held under known and controlled conditions will be discussed in another paper.

SUMMARY

A study has been made of 486 cultures of bacteria isolated from butter made and held under known and controlled conditions. Only those organisms capable of hydrolyzing milkfat, or splitting tributyrin, or digesting casein have been included.

Of the cultures studied, 39 per cent (188) were gram negative rods; 31 per cent (151) were gram positive cocci; 15 per cent (74) were gram positive, aerobic, sporeproducing rods; 8 per cent (40) were gram negative micrococci; 5 per cent (23) were gram positive sarcina; and 2 per cent (10) were gram positive nonsporeproducing rods.

Those groups occurring in numbers large enough to be of probable significance in the spoilage of butter were gram negative rods, gram negative micrococci, gram positive sarcina, and gram positive nonsporeproducing rods.

In the butters supporting the growth of large numbers of bacteria, only the gram negative rods were regularly present in large enough numbers to be of importance in the spoilage of all of the butters tested.

New names have been suggested for five species of bacteria: *Micrococcus intermedius*, *Micrococcus lipolyticus*, *Micrococcus magnus*, *Micrococcus tributyrus*, and *Sarcina caseolytica*.

Data have been presented to show the importance of the use of milk agar as an indication of the keeping quality of butter.

A SUMMARY OF THE MORPHOLOGICAL AND PHYSIOLOGICAL PROPERTIES OF THE NEW SPECIES STUDIED

Micrococcus intermedius

Spheres: when old. Oval and rod-like when young. Occurring singly, in pairs and irregular masses. Gram positive.

Gelatin stab: No liquefaction.
Agar colonies: Large, moist, light-yellow colonies with dark centers; round, entire margin.
Agar slant: Light-yellow, moist, abundant growth with raised margin.
Broth: Turbid, yellow sediment.
Litmus milk: Alkaline; no curd, no proteolysis, no reduction.
Milkfat is hydrolyzed.
Tributyrin is hydrolyzed.
Indol not formed.
Nitrates not reduced.
No acid in dextrose, lactose or sucrose.
Ammonia is produced.
Aerobic.
Grows generally at 10° C. and 37° C. Some may grow as low as 5° C. and some as high as 45° C. Does not grow at 50° C.

Micrococcus lipolyticus

Spheres: Occurring singly and in irregular clumps. Gram negative.
Gelatin stab: No liquefaction.
Agar colonies: Medium, white, moist, round; serrate margin.
Agar slant: White, thick, moist growth.
Broth: Turbid, white sediment.
Litmus milk: Alkaline; generally slight reduction; no curd, no proteolysis.
Milkfat is hydrolyzed.
Tributyrin is hydrolyzed.
Indol not formed.
Nitrates not reduced.
No acid in dextrose, lactose, or sucrose.
Ammonia is produced.
Aerobic.
Grows at 5° C. and 37° C. and may grow at 40° C. Does not grow at 45° C.

Micrococcus magnus

Spheres: 2 to 3 microns in diameter. Occurring singly and in irregular clumps. Gram positive.
Gelatin stab: No liquefaction.
Agar colonies: White, moist, small to medium, irregular; depressed center with raised margin.
Agar slant: White, moist, rough, scanty growth.
Broth: Turbid, white sediment.
Litmus milk: Acid and slight reduction; generally curdled; no proteolysis.
Milkfat is hydrolyzed.
Tributyrin is hydrolyzed.

Indol not formed.
Nitrates reduced to nitrites.
Acid from dextrose, lactose and sucrose.
Ammonia is produced.
Aerobic.
Grows at 10° C. and 45° C. Does not grow at 5° C. and 50° C.

Micrococcus tributyrus

Spheres: 1.5 to 2.5 microns in diameter. Occurring singly or in irregular clumps. Gram positive.
Gelatin stab: Liquefaction.
Agar colonies: Large, yellow, moist, round; thin irregular margin.
Agar slant: Light-yellow, thick, moist, raised.
Broth: Turbid, yellow sediment.
Litmus milk: Acid, curd, digestion, reduction.
Milkfat not hydrolyzed.
Tributyrin is hydrolyzed.
Indol not formed.
Nitrates reduced to nitrites.
Acid from dextrose, lactose, and sucrose.
Ammonia is produced.
Aerobic.
Grows generally at 10° C. and 40° C., and may grow as high as 50° C. and as low as 5° C.

Sarcina caseolytica

Spheres: 1 to 1.5 microns in diameter, grouped in packets of eight. Gram positive.
Gelatin stab: Liquefaction.
Agar colonies: White, moist, medium to large; raised center, irregular margin.
Agar slants: White, moist, rough, scanty growth.
Broth: Slight turbidity, little sediment.
Litmus milk: Curd, digestion, reduction; no acid.
Milkfat not hydrolyzed.
Tributyrin not hydrolyzed.
Indol not formed.
Nitrates reduced to nitrites.
Acid from sucrose; no acid in dextrose or lactose.
Ammonia is produced.
Aerobic.
Grows at 10° C. and 45° C. Does not grow at 5° C. and 50° C.

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THE FLUOROMETRIC ESTIMATION OF LACTOFLAVIN

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Knowledge concerning the nature of the components of the vitamin B-complex has been extended by the comparatively recent studies of various authors. Many investigators (1–14) have called attention to the growth-promoting properties of pure lactoflavin, a yellow-red pigment which has been isolated from such products as whey (15, 16), egg (17, 18), liver (19, 20), yeast (21) and hay (22). Lactoflavin exhibits a marked greenish-yellow fluorescence, especially when subjected to the visible blue-violet rays (23). The fluorescent characteristics of impure lactoflavin concentrates, prepared from a water-soluble vitamin concentrate derived from milk, have been correlated with the growth-promoting properties of such concentrates (24). These studies as well as others of a similar character have indicated the possibility of utilising the fluorescent properties of lactoflavin as a basis for the qualitative and quantitative estimation of this dietary factor. In order to determine the merits and limitations of such a procedure, pure lactoflavin must be used as the basis of study. Crystalline lactoflavin, isolated from the previously mentioned water-soluble vitamin concentrate derived from milk, has been employed for determining its fluorescent characteristics in the longer, invisible ultra-violet rays. The following experimental data, illustrated by photographic reproductions in true color of the "black light" fluorescence, indicate the value of the fluorometric method for the estimation of lactoflavin.

EXPERIMENTAL

Pure, crystalline lactoflavin was isolated from the crude milk vitamin concentrate (25) by a series of adsorption and elution procedures which resulted in impure concentrates of a relatively high lactoflavin content. Such concentrates were further purified by the formation of the insoluble silver salt and its decomposition with hydrogen sulfide which yielded a lactoflavin solution from which the pure substance was recovered by further adsorption and elution procedures followed by recrystallizations from alcohol. An amount of 1.2 gm. of pure lactoflavin was recovered from 32 kg. of the crude milk vitamin concentrate; such a recovery is several hundred times higher than the yields which other workers (15, 16, 21, 26, 27) obtained from liquid whey.

The crystalline character of the pure product is illustrated in the accompanying plates IB and I. In determining its melting-point it was observed that a change of state, indicative of melting and decomposition, took place

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within the temperature range of 267–294° C. Kuhn, Rudy and Weygand (28) have reported identical observations with crystalline lactoflavin and record a "melting point" of 293° C. (uncorr.)

The pure product, when dissolved in water shows a yellow fluorescence with a greenish cast in natural light if the concentration is sufficiently high. The fluorescence appears markedly intensified, when such solutions are examined in the longer invisible ultra-violet rays. This intensified fluorescence in "black light" seemed to indicate that it might be used as a basis of a method for the quantitative estimation of lactoflavin in solution. In view of its convenience and the character of its emissions an Eveready Fluoray lamp[1] was used in the development and application of the fluorometric method described hereinafter.

Preparation of Standard Lactoflavin Solutions: 25.473 mg (± 0.005 mg.) of the pure, crystalline lactoflavin were dissolved in a sufficient amount of water to obtain a stock solution which contained 250 γ /ml. of lactoflavin. Solutions of a known lactoflavin content were prepared by diluting appropriate samples of this stock solution with water; 5 ml. portions of these solutions were introduced into non-fluorescent glass vials, one drop of a 37 per cent formaldehyde solution added and the vials closed with cleaned stoppers. The range of concentrations prepared varied between 25.00 and 0.10 γ/ml. of lactoflavin. These standard solutions had a pH sufficiently close to the neutral point to assure the maximum fluorescence (29, 30).

Description of Light Source: The Eveready Fluoray lamp is a portable carbon arc lamp burning C carbon electrodes at 30 volts and 9 amperes (31) which emit a substantially continuous spectrum throughout the longer ultra-violet light range. The arc is entirely shielded except for a small opening which permits the passage of a small beam of light. The lamp is equipped with a molded, 4 mm. thick, heat-resisting red purple ultra filter No. 587.[2] This filter transmits only radiations between about 3100 and 4100 Å and very slightly in the extreme red (about 7200 Å); its peak transmission is at about 3650 Å.

Examination of the Lactoflavin Standards: Examination of the lactoflavin solutions in "black light" should be made in an appropriate room from which all light except that emitted by the lamp is entirely excluded. Preferably the examination should be made against a black, non-fluorescing and non-reflecting background. Under such conditions the standard solutions exhibit a brilliant yellow fluorescence with a greenish cast, as reproduced in the accompanying plates II and III. The quality of the fluorescence appears to be the same in all the standard solutions, but, in the accompanying plates, the standard solutions of lower concentrations seemed to have an

[1] The Eveready Fluoray lamp was obtained through the courtesy of The National Carbon Company, Cleveland, Ohio.

[2] This filter is manufactured by the Corning Glass Works, Corning, New York.

increased greenish cast; this is probably due to the fact that the standard solutions were photographed against a black background. The intensity of the fluorescence, however, decreases with decreasing lactoflavin concentrations within the 25–0.1 γ/ml. range and it seems that the rate of decreasing intensity increases with decreasing concentrations. The relative fluorescence intensity of a 50 γ/ml. and a 25 γ/ml. solution appeared not to be sufficiently different to be used for analytical purposes. The lower range of concentrations, as e.g. 2.5–0.1 γ/ml. of lactoflavin, however, did show contrasting differences in fluorescence intensity suitable for a fluorometric comparison. The extinction point was found to be a concentration of about 0.05 γ/ml. of lactoflavin.

Parenthetically it should be noted that examination of the standard solutions in the light of the unscreened arc showed that solutions containing less than 1 γ/ml. of lactoflavin do not exhibit a fluorescence detectable with the naked eye. However, von Euler and Adler (32) were able to examine solutions containing as little as 0.02 γ/ml. of lactoflavin in the condensed light of an unscreened carbon arc. When observed in natural light, the solutions of higher concentrations appear to be yellow and those of lower concentrations practically colorless.

Estimation of Pure Lactoflavin in Solutions of Unknown Concentrations: Lactoflavin solutions of unknown concentrations were compared in "black light" with the standard solutions for the purpose of ascertaining the reliability and limitations of the method. Fractions of such unknown solutions, which upon preliminary examination in "black light" were estimated to contain in excess of 25 γ/ml. of lactoflavin, were diluted with water to make a series of concentrations which showed a fluorescence intensity comparable to standard solutions containing less than 2.5 γ/ml. After calculating the concentration of lactoflavin by reference to the standard solutions and then referring to the concentration of the original sample, known to other individuals but withheld from the analyst until after the determination by the fluorometric method had been completed, it was found that a degree of accuracy within 0.1 γ of lactoflavin could be obtained readily.

Estimations of Lactoflavin in Impure Concentrates: The above method of estimation is not only applicable to solutions of pure lactoflavin but also to lactoflavin concentrates containing a relatively high amount of impurities, provided that the fluorescent color is not interfered with by the impurities. This interference may frequently be overcome by proper dilution. The method was applied, for example, to a lactoflavin containing elution product obtained by treating a fullers' earth adsorbate with dilute pyridine [Sample 2-550 (24)]. This sample contained 33,500 γ of total solids per ml., much of which was extraneous matter other than lactoflavin. The sample was diluted to different concentrations varying from 33.55 γ/ml. to 5.03 γ/ml. of total solids. These dilutions which appeared practically colorless in day-

light were examined in "black light" in comparison with the standard solutions. It was found that the fluorescence intensity of the 26.84 γ/ml. concentration matched with that of the 0.50 γ/ml. standard lactoflavin solution; the fluorescence intensity of the 13.42 γ/ml. concentration matched with that of the 0.25 γ/ml. of standard lactoflavin solution; the fluorescence intensity of the 0.10 γ/ml. standard lactoflavin solution was slightly lower than that of the 6.71 γ/ml. concentration and slightly higher than that of the 5.03 γ/ml. concentration. From these data it was calculated that the unknown sample (2-550) contained 620.68 γ of lactoflavin per ml. or per 33,500 γ of total solids.

The above example clearly shows the applicability of the method particularly when the examination of the unknown sample is made in comparison with pure lactoflavin solutions within the 0.5–0.1 γ/ml. range.

DISCUSSION

The above data as a whole seem to show that the fluorometric method is accurate and can be applied to pure lactoflavin solutions and to lactoflavin concentrates containing relatively high amounts of impurities. The method was found to be very useful in the process of isolation of lactoflavin from various sources; it permits e.g. ascertaining the efficiency of adsorbents and eluting agents employed in the quantitative recovery and purification of lactoflavin.

The method was found to be applicable also for obtaining relative approximations of the lactoflavin content of such biological materials as e.g. dried liver, spinach, milk, whey, crude milk sugar, etc. The applicability of the method for estimating the lactoflavin content of such material depends upon the completeness with which the lactoflavin can be extracted by various solvents; acetone in proper dilutions has been found to be very convenient when applied to certain biological materials.

Various data which will be reported in subsequent papers seem to show conclusively that comparable and consistent results can be obtained by the fluorometric method described. The results obtained with the fluorometric method were correlated with the biological data concerning the growth-promoting properties of lactoflavin which now appear to serve as further confirmatory evidence substantiating the merits of the fluorometric method as an adjunct facilitating biological investigations involving this dietary factor.

SUMMARY

Pure, crystalline lactoflavin has been prepared from a water-soluble vitamin concentrate derived from milk. A method of estimating lactoflavin involving its fluorescent properties in "black light" is presented. The method is simple, involving only standard lactoflavin solutions and an ultra-

violet light generator equipped with a suitable filter. The method was found to be accurate to 0.1 γ of lactoflavin, but concentrations as low as 0.05 γ/ml. of lactoflavin could be detected.

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PLATE IB.
Crystalline Lactoflavin from Milk Vitamin Concentrate.
(Magnification X 60)

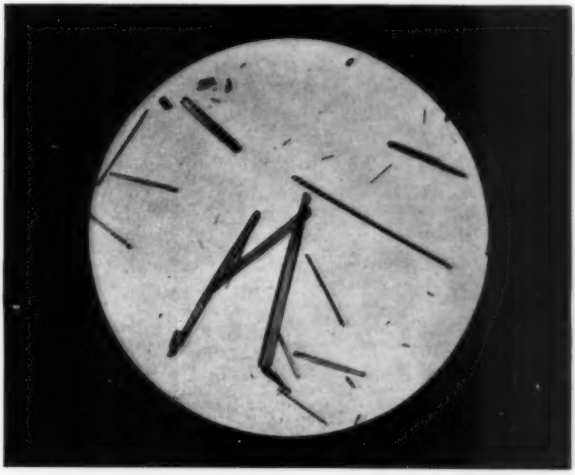

PLATE I.
Crystalline Lactoflavin (natural color) from Milk Vitamin Concentrate.
(Magnification X 60)

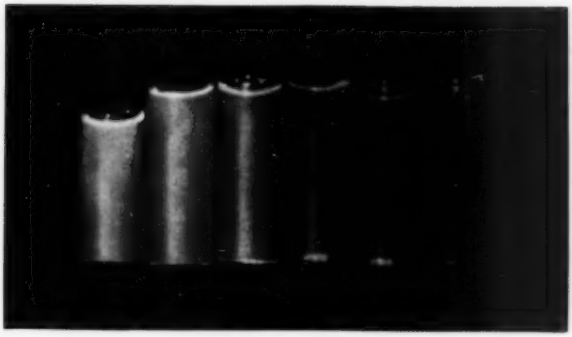

PLATE II.

Fluorescent Color of Pure Lactoflavin in "Black Light".

Tube 1—25 gamma per cc.
Tube 2—15 gamma per cc.
Tube 3—10 gamma per cc.
Tube 4— 5 gamma per cc.
Tube 5— 2.5 gamma per cc.
Tube 6—Distilled Water.

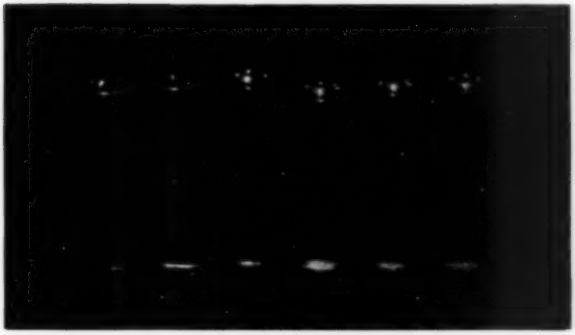

PLATE III.

Fluorescent Color of Pure Lactoflavin in "Black Light".

Tube 1—1.50 gamma per cc.
Tube 2—1.00 gamma per cc.
Tube 3—0.50 gamma per cc.
Tube 4—0.25 gamma per cc.
Tube 5—0.10 gamma per cc.
Tube 6—Distilled Water.

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ABSTRACTS OF LITERATURE ON MILK AND MILK PRODUCTS

BUTTER

Effect of temperature, salt and acidity on growth of mould (Oospora lactis). H. MACY AND A. E. ANDERSON, Division of Dairy Husbandry, Univ. of Minn., St. Paul, Minnesota. National Butter and Cheese Jour. Vol. 25, No. 22, Nov. 25, 1934.

A study was made of the conditions affecting the growth of the mould (*Oospora lactis*), which is the most prevalent species found in milk and dairy products. Seven cultures were isolated from butter and their ability to grow on substrates containing varying amounts of salt, varying acidities, and at different temperatures was observed. The substances used were whey agar, and buttermilk obtained from the churning of sweet and sour cream.

Growth was extensive in controls on all media at 20° C. (68° F.), less luxuriant at 10° C. (50° F.) and 2° C. (35.6° F.), and entirely checked at –23° C. (9.4° F.). Low temperatures had no permanent effect on the viability of the cultures except when the pH of the whey agar was low.

The growth on whey agar was affected when the salt concentration exceeded 1 per cent and was entirely checked at 10 per cent; the growth on buttermilk was usually retarded at a salt concentration above 2.5 per cent and stopped at 10 per cent.

The degree of acidity did not in itself have a very marked effect on the extent of growth. The combined effects of high salt concentration and low temperature and/or hydrogen ion concentration were noticeable.

CHEESE

Disturbances in the natural oxidation-reduction equilibrium of milk with special reference to the use of the dehydrated milks in the manufacture of cottage cheese. W. H. E. REED AND R. L. BROCK, Dairy Dept., Missouri Agr. Exp. Sta., Columbia, Missouri. Missouri Agr. Exp. Sta. Res. Bull. 216, July, 1934.

This bulletin is a technical study of certain phases of the use of dry milk in the manufacture of cottage cheese.

CONDENSED AND DRY MILK

Evaporated milk and beta lactose in infant feeding. SIMON SKOLE, Med. Rec. 139: 87, Jan. 17, 1934.

A group of 54 infants fed evaporated milk and beta lactose showed better growth and health than did a control group of 54 on ordinary fluid

milk and carbohydrate formulas. The uniform composition and highly digestible fat and protein of evaporated milk are important factors in the success of this form of milk as an infant food. Lactose, being the same carbohydrate as that which occurs in breast milk, is more slowly and more completely assimilated than are other carbohydrates, and fermentation occurs in the small intestine, thereby preserving the normal intestinal flora.

The group of infants receiving evaporated milk and beta lactose showed better weight gain, better tissue turgor, less constipation, and fewer gastro-intestinal upsets than did the control group. No case of rickets occurred in the evaporated milk group. The author suggests the superior nutrition of this group as an important factor in the prevention of rickets, possibly more important than the administration of any form of vitamin D.

The relation of dry skim milk to several of the physical and chemical properties of cream cheese. W. H. E. Reid and H. R. Alley, Dairy Dept., Missouri Agr. Exp. Sta., Columbia, Missouri. Missouri Res. Bull. 213, July, 1934.

This investigation deals with the manufacture of cream cheese by recently developed procedures with special reference to the use of dry skim-milk.

The use of evaporated milk in digestive disorders, particularly peptic ulcer. P. B. Davidson, F. Biguria and Ruth Guild. Jour. Am. Diet. Assn., 9: 478, March, 1934.

The value of milk and cream in the treatment of gastro-intestinal disorders lies in their high nutritive value, their ability to inhibit gastric secretion, and their high acid-combining power.

As a result of studies on peptic ulcer patients, in order to determine the comparative stimulating power of fluid milk, evaporated milk, diluted and undiluted, and milk with 20 per cent cream added, the authors conclude that undiluted evaporated milk and milk and cream are both less stimulating than diluted evaporated milk or fluid milk. The influence of all four forms of milk on gastric acidity and motility were found to be practically the same.

Evaporated milk in infant feeding. A clinical study of 400 cases. Warren Quillian. Jour. Fla. Med. Assn., 20: 291, Jan., 1934.

Evaporated milk and beta lactose formulas were given successfully to three groups of infants, and the results compared favorably with those observed in infants receiving other formulas. The uniformity of composition, superior digestibility, and simplicity of evaporated milk are important considerations, especially when the purity and safety of the fluid milk supply is questionable and the economic status and intelligence of the mother are not high. The first group of infants in a city clinic, the second

group of premature babies in a hospital and the third group taken from private practice also showed excellent growth and freedom from constipation and diarrhea after the formulas were properly adjusted. A total of 340 cases were involved.

MILK

The color imparted to coffee by cream treated in various ways. RANDALL WHITAKER. Res. Lab., National Dairy Prod. Corp., Baltimore, Maryland. Jour. Dairy Science Vol. 17, No. 10, p. 651, Oct., 1934.

It was observed by the author and reported by consumers that bottled sweet creams of competing companies varied in their ability to color coffee. A method of measuring the coloring power of cream in coffee was devised. It consisted in the preparation of a standard coffee solution prepared by swirling a cheese-cloth bag containing 2.2 pounds of medium ground coffee for 5 minutes in 5 gallons of water at 200° F. The coffee was then filtered and sterilized in 1 pint or 1 quart bottles for 25 minutes under 15 pounds steam pressure and stored at room temperature until required. In making the test 100 cc. of the coffee solution at 180–200° F. was poured into a white cup containing 10 cc. of the cream at 40–45° F. The color was then matched a standard golden yellow-brown of the Munsell system. If the color did not match the test was repeated using more or less cream until the desired shade was secured.

It was found that coffee cream in the Baltimore area required from 7.7 to 10.6 cc. to give the desired color while whipping cream required from 7.1 to 8.5 cc. Furthermore, cream from the same plant may vary from day to day. The amount of cream required to impart to coffee a given color decreased when the fat content increased (10.0 cc. for 20 per cent cream and 5.7 cc. for 40 per cent cream), when the solids-not-fat content increased (an additional one per cent of solids caused the required cream to decrease from 10.0 to 9.0 cc.) and when the cream was homogenized (this decrease was 0.6 cc. for cream homogenized at 400 pounds pressure.

Pasteurization of cream and aging had no effect upon the quantity required to develop a standard color in coffee. When cream was heat treated to 86° F. to increase its viscosity the amount required to give comparable colors in coffee was increased from 10.0 to 10.3 cc.

Some essentials in quality milk production. J. G. HARDENBURGH, Walker-Gordon Co., Plainsboro, N. J. Certified Milk Vol. 9, No. 102, 9–15, October, 1934.

During the past four decades, the dairy industry has made greater advances in this country than it has in most countries during the past four centuries. The production of good milk is the most complicated branch of the farming industry and requires a knowledge of animal husbandry,

of feeds and their utilization by the cow, of animal physiology, of the fundamental principles of hygiene as applied to the control of animal diseases, as well as of the sanitary requirements in the production of a highly perishable food.

The essential requirements for the efficient operation of a dairy business are becoming so much more and more technical that it is tending inevitably to centralize the production into fewer and larger units. Economic handling of a dairy herd demands that the herd be of sufficient size to support the overhead expense of adequate modern machinery and the expense involved in the selection and elimination of diseased animals. Farmers who are milking a few cows as a sideline cannot afford to follow the rigid schedule of sanitary control measures involving periodic examination of cows, and many other items prerequisite to adequate herd management.

Milk production per square mile offers interesting study. E. C. DAMROW, Damrow Brothers Co., Fond du Lac, Wisconsin. National Butter and Cheese Jour., Vol. 25, No. 15, p. 14, Aug. 10, 1934.

The 100 leading milk producing counties in this country are listed, according to the United States Department of Commerce, Bureau of Census Report for 1930 (see accompanying table).

Out of the 100 counties listed in 12 states, Wisconsin has 37 counties, New York 23, California and Minnesota 10 each, Pennsylvania 8, Illinois 3, Iowa, Vermont, and Washington 2 each, and Massachusetts, Michigan and New Jersey 1 each.

Shaded maps of the states of Wisconsin and New York are presented.

The value of hand stripping after machine milking. JOHN L. WILSON AND C. Y. CANNON, Iowa Agr. Exp. Station, Ames, Iowa. Jour. Dairy Science, Vol. 17, No. 4, p. 331, April, 1934.

Stripping versus not stripping after machine milking was studied in a double reversal trial with 40-day periods. During periods in which the cows were stripped after the milking machine the production of milk and butterfat was 2.5 per cent greater than in periods in which stripping was omitted. Calculations show that not stripping resulted in the loss of 54 per cent of the milk and 27 per cent of the fat which would have been present in the strippings had the cows been stripped. The fat percentage in the milk was not influenced by omission of stripping.

Practices designed to replace hand stripping were only partially effective. Massaging the udder during the last two minutes the cows were being milked by the machine reduced the amount of strippings 33 per cent. Manipulation (pulling down) of the test cups during the last minute of milking caused a 55 per cent reduction in the amount of strippings.

The fat percentage of milk as affected by feeding fats to dairy cows. NAT. N. ALLEN, Div. of Dairy Husbandry, Univ. of Minn., St. Paul, Minn. Jour. Dairy Science Vol. 17, No. 5, p. 379, May, 1934.

The author has shown that the butter fat content of milk may be appreciably increased for short periods of time by feeding grain rations enriched with fat.

A survey of certified milks. DAVID WILBUR HORN. Lecturer in Hygiene, Hahnemann Medical College, Bryn Mawr, Pa. The Hahnemannian Monthly, Vol. LXIX, No. 6, p. 401, June, 1934.

During the past 10 years the author has examined 2,248 samples of certified milk. The samples were purchased by health officers as the milk was being sold to customers or was being carried on delivery wagons. The samples were iced immediately and delivered to the laboratory. These samples were taken in the suburban area of Philadelphia. The samples were collected from 1923–1932 inclusive, with considerable monthly and yearly uniformity.

The bacterial counts made by the standard plate method were grouped on the basis of all counts in the hundreds, all counts in the thousands, etc., and these counts were then grouped by months.

There were 361 samples with counts below 1000, there were 1,578 samples with counts between 1,000 and 10,000, and 52 samples with counts above 100,000. Although many samples seemed to be rather high in count for this grade of milk, it should be borne in mind that 90 per cent of all samples contained not more than 10,000 colonies per cc. which is the standard for certified milk.

Analysis for butterfat showed that probably 95 per cent of all samples met the standard of an average of 4.0 per cent fat and a minimum of 3.5 per cent.

Based upon the usual laboratory tests there were a number of irregularities other than in bacterial counts and butterfat composition which were classified as follows:

Offence	Number of offences
Visible dirt	203
Skimming	142
Added water	2
Low total solids	3
Excessive leucocytes	9
Misbranding	4

Schardinger reaction on raw colostral milk and hygienic control of milk for consumption. A. HOUDINIER, Food Inspector at Nancy. Le Lait, 14, 468–78, 591–603, 1934.

The time of the Schardinger reaction varies with temperature and quantity of reagent used. Normal milk causes a reduction in 10 to 15 minutes at 40 to 65° F., while colostral milk does not decolorize the methylene blue in 48 hours. Mixtures of colostral and normal milk give a retarded action. This method may also be employed to detect high temperature pasteurization. High temperature pasteurization retards the reduction of methylene blue. The use of the Schardinger reaction for hygienic control is therefore of value where high pasteurization temperatures are not employed.

Studies on acidophilus milk. C. N. Stark, Ruth Gordon, J. C. Mauer, L. R. Curtis, and J. H. Schubert, Cornell University, Ithaca, N. Y. Amer. Jour. Pub. Health, Vol. 24, No. 5, p. 470–472, 1934.

In an attempt to evaluate the therapeutic value of acidophilus milk, the fecal contents of 124 persons were examined over a period of 18 months. Of the 124 patients, 66 were constipated and 58 were considered normal in this respect. All of the persons examined were found to have on the average approximately 1000 lactobacilli per gram of their feces. During the time the patients consumed acidophilus milk, approximately 35 per cent of the fecal samples contained more than ten million lactobacilli per gram, and another 35 per cent contained in excess of one hundred million. These acidophilus types soon disappeared after the acidophilus milk was discontinued even though the patients continued to drink milk containing 3 per cent of added lactose.

Analyses of fecal samples before, during, and after acidophilus therapy showed (1) that there was little change in the number of non-sporulating gas producing colon types of bacteria in the fecal flora, (2) a definite tendency for the number of streptococci to decrease during the period of therapy, especially in the non-constipated group, (3) the number of spore forming anaerobes such as *Clostridium Welchii* tended to decrease during treatment, (4) there was a definite decrease in the amount of indol both in the feces of group fed acidophilus milk and the group fed sweet milk plus 3 per cent of lactose, (5) there was no appreciable change in the pH of the fecal contents during treatment, (6) the moisture content of the feces increased during the period of acidophilus therapy in both the constipated and non-constipated groups, but after discontinuing the treatment the moisture content of the feces from the constipated group soon reverted to the original low value, whereas that from the non-constipated group remained high, (7) of the 74 persons who completed the experiments, 29 of the 43 who were in the constipated group felt they had been definitely helped. A majority of the non-constipated group felt that they were in much better physical condition while they were drinking acidophilus milk.

Special milks. Report of the committee on milk and dairy products. Year Book, Amer. Public Health Assn., 1933–1934, p. 63.

The outstanding progress in the past year in the methods of production of vitamin D milk, thus providing a sure and simple control of rickets and other conditions arising from a lack of vitamin D, is reviewed and discussed. Certain difficulties remain to be overcome, however, and these include a suitable standard by which the potency of milk may be measured and adequately marked on the bottle. As matters stand now the various methods of providing milk with additional vitamin D result in products of varying potency as measured by the Steenbock rat unit, irradiated milk supplying protection in 35 to 42 rat units, yeast milk in about double that number. The addition of cod liver oil concentrate requires an even greater number of rat units for an equal degree of protection. Further study is necessary to clear up these differences and arrive at a uniform measure of potency before legal standards can be set up. The question whether vitamin D milk is a food or a drug is discussed.

The place of homogenized milk in infant feeding is still very much in the experimental stage. It has been suggested that homogenized milk has a higher hydrogen-ion concentration. When the milk has not been previously heated higher than 120° F. this change in pH occurs almost immediately; a higher preheating causes the pH not to change for from 12 to 24 hours. Such milk spoils rapidly. The influence of homogenization on the vitamin content of milk should be further investigated, in the opinion of the committee.

A study of the effect of preserving methods on human milk. W. H. Eddy and S. G. Morris. Jour. of Ped., 4: 208, Feb., 1934.

Human milk collected for distribution when needed and preserved either by dehydration or by freezing has been examined for the effect of these processes on the nutritive value of the milk, in comparisons with fresh human and fresh cow's milk. The results indicate that freezing is an entirely satisfactory method of preservation, its effect on vitamins A, B and G being practically negligible. Some destruction of vitamin A was observed as a result of dehydration. Vitamins C and D were not present in either the fresh or preserved human milks in sufficient quantities to warrant omitting the usual supplementary orange juice and cod liver oil.

A study of breast milk fat. P. E. Roller. Jour. Ped., 4: 238, Feb., 1934.

In a study of the water-insoluble acids in the fat of breast milk a comparison with those of cow's milk is given, showing that breast milk has a higher value for oleic, linoleic and palmitic acids, but that cow's milk is higher in other lower saturated acids.

Rapid methods for the quantitative determination of total protein and non-protein nitrogen in human and cow's milk. LESLIE DOUGLAS SCOTT, Charing Cross Hospital, Inst. of Pathology, London, England. Biochem. Jour. Vol. 28, No. 4, p. 1193, 1934.

This method is designed not to determine the total nitrogen content of milk but the true total protein content. The method used is as follows: To 10 milliliters of 0.45 per cent zinc sulphate in a 15 milliliter centrifuge tube add 1 milliliter of human or 0.50 milliliter of cow's milk. Mix well and add 2 milliliters of N/10 sodium hydroxide. After mixing centrifuge at 2000 r.p.m. for 5 minutes carefully pour off supernatant liquid and repeat process, this time using 10 milliliters of zinc sulfate solution and 1 milliliter of N/10 NaOH. With the use of as little water as possible transfer the precipitate to a digestion flask and add 5 milliliters of a digestion mixture of 50 milliliters 10 per cent copper sulphate, 300 milliliters of 85 per cent phosphoric and 100 milliliters of nitrogen free sulphuric acid. When digestion is complete transfer contents to a 250 milliliter flask and make up to volume. Nesslerize (Folin and Wu J. Biol. Chem 38: 81, 1919) 50 milliliters of this solution in a 100 milliliter graduated flask and dilute to volume. Compare the color produced to that of a Nesslerized unknown solution prepared from 3 milliliters of a standard ammonium sulphate solution (.494 g. per liter) and 1 milliliter of the digestion mixture in a 100 milliliter graduated flask.

$$\frac{\text{Standard}}{\text{Unknown}} \times 157 = \text{Mg N/100 ml. human milk}$$

$$\text{This result} \times \frac{6.38}{1000} = \text{per cent of protein in human milk}$$

$$\text{Final result} \times 2 = \text{protein in cow's milk}$$

The results obtained by this method have been compared with those obtained with the tungstic acid precipitation method and the results have been found to agree very well.

Cost of production of market milk for Los Angeles. JOHN MARSHALL, JR., Div. of Markets, Calif. State Dept. of Agr., Sacramento, California. Pacific Dairy Review, Vol. 38, No. 10, p. 12, Oct., 1934.

The 74 producers from whom complete records were obtained, produced and sold for distribution as fluid milk, 3,308,836 pounds of butterfat during the year 1933, or approximately 16 per cent of the total amount of milk distributed in the Los Angeles sales area. Fifteen of the producers were tenants, and 59 owned part or all of the dairy farm operated. The average size of the farms was 61.80 acres, of which 19.51 acres were actually used by the dairy.

The average price received by the average producer in this area for the year 1933 was approximately 46.5 cents per pound butterfat. The average

cost of producing a pound of butterfat was 53 cents being made up as follows; rent 2.5, equipment 1.2, herd maintenance 3.1, feed 27.6, labor 13.1, operating 4.6, hauling 2.9 and from these expenses 1.9 cents was deducted for miscellaneous income.

Rate of growth and acid production of Streptococcus lactis. J. M. SHERMAN AND H. M. HODGE, Cornell Univ., Ithaca, N. Y. Jour. Dairy Science Vol. 17, No. 7, p. 497, July, 1934.

Evidence has been published to show that slow growth permits adaptation of an organism to its environment and hence the development of more resistant organisms. On this basis slow growing cultures should develop the more acid milk cultures. To test this hypothesis sterile milk was inoculated with *Streptococcus lactis* in varying amounts with the thought that the great dilutions would have only rapidly growing organisms which could not resist high acidity. In a general way it was found that very low inoculations reduced the final acidity developed in the culture. Again, if cultures are transferred at longer time intervals the effect of dilution in the inoculated milk should not exist as the slow growing organisms had time to develop. This was found to be true.

The effect of heat and chemical sterilization on the rubber parts of milking machines. J. L. HENDERSON, C. L. ROADHOUSE AND A. FOLGER, Univ. of California, Davis, California. Jour. Dairy Science Vol. 17, No. 7, p. 475, July, 1934.

Data have been secured on the life of rubber parts of milking machines and their sterility as a result of sterilization by hot water, chlorine solutions, and lye solutions.

It was found that heating rubber parts in water at 185° F. for 20 minutes was much more effective as a sterilizer than 170° for 20 minutes and did not appreciably shorten the life of the rubber. Both heat treatments reduced the lifetime of the rubber about one-third. A solution of 200 p.p.m. of chlorine and 0.3 or 0.5 per cent of sodium hydroxide gave slightly higher counts than heat sterilization. With chlorine solutions the life of the rubber was more dependent upon age than upon usage.

The differentiation of Streptococcus lactis from Streptococcus fecals. J. M. SHERMAN AND PAULINE STARK, Cornell Univ, Ithaca, N. Y. Jour. Dairy Science Vol. 17, No. 8, p. 525, Aug., 1934.

There is some question concerning the identification of *Streptococcus lactis* and *Streptococcus fecalis* as the two species are so nearly identical.

The authors have used four tests by which these two groups may be readily differentiated. (1) None of the lactis cultures grow above 45° C. while fecalis has its optimum temperature at 48–52° C. (2) All cultures of lactis in skimmilk were killed at 65° C. for 30 minutes while all cultures

of fecalis survive. (3) When seeded in lactose nutrient agar fecalis grows rapidly at pH 9.6 whereas lactis is entirely inhibited. (4) Cultures of *Streptococcus lactis* were all inhibited by 6 per cent salt while fecalis grew well in 6.5 per cent salt solutions.

A new method for converting average whole milk into soft curd milk. ERIK LUNDSTEDT, Pfister Chem. Company, Ridgefield, N. J. Milk Plant Monthly, Vol. 23, No. 7, 1934.

Human milk is easier to digest than is cow's milk. The reason is that mother's milk forms fine curds; whereas cow's milk forms large hard curds. When cow's milk is diluted with water it is easier to digest than in its normal state, but basically the nature of the curd remains unaltered. Dilution tends to retard digestion.

The curd tension is decreased about 30 per cent when milk is boiled. Adding 3 per cent of lemon juice or 8 per cent of orange juice renders the average milk more digestible. It also increases the vitamin C potency of the milk.

The Hill curd method is described. Milk ranges from 5 to 200 Hill grams in curd tension. The average milk in the U. S. A. ranges from 50 to 70. A range of 5 to 30 is standard for soft curd milk.

Homogenizing milk at 40 to 45° C. (104 to 113° F.) at 300 or more pounds of pressure will decrease the firmness of the curd. About 25 per cent of the casein is adsorbed on the fat from the serum as a result of homogenization.

Curd can be softened by passing milk through Zeolites removing 20 per cent of the calcium. This necessitates an increased consumption demand since 6 units in place of 5 of milk are required to give equal calcium values.

Influence of mastitis on the curd tension of milk. H. C. HANSON, D. R. THEOPHILUS, F. W. ATKESON, AND E. M. GILDOW, Idaho Agr. Exp. Station, Moscow, Idaho. Jour. Dairy Science, Vol. 17, No. 3, p. 257, March, 1934.

Composite samples of milk from 26 Holstein and 20 Jersey cows were examined for bacterial content, special types of bacteria, leucocytes and for hardness of curd. Some samples from individual quarters were also studied. The udders of the cows were examined physically for mastitis.

Consideration of the data showed that the usual streptococci mastitis lowered the curd tension of milk while staphylococci infections had no effect. Classification of orders on the basis of fibrous tissue gave no relationship to the curd tension of milk.

The authors believe that undue emphasis has been given to the possibility of mastitis producing soft curd milk. This is particularly very improbable in dairies which have veterinary supervision.

Outbreaks of milk poisoning due to a toxin-producing staphylococcus found in the udders of two cows. JAMES A. CRABTREE AND WILLIAM LITTERER, Tenn. State Dept. of Health, Nashville, Tennessee. Amer. Jour. Pub. Health, Vol. 24, No. 11, p. 1116–1122, Nov., 1934.

A series of outbreaks of gastro-enteritis among the students and faculty in a boarding school showed a definite periodicity of 7- to 10-day intervals. There were 242 cases among the 97 persons, 12.4 per cent of them having as many as 5 reoccurrences. Chemical and bacteriological analyses of all foods gave negative results except in the case of milk. Hemolytic staphylococci were isolated from the milk served as well as from the throats and the vomitus of those suffering from the malady. Of the 242 cases, all but one occurred in persons who had drank milk during the meal immediately preceding the onset. The filtrates prepared from the isolated cultures of staphylococci contained a large amount of toxic substance. Human volunteers who consumed milk containing only 3 cc. of this filtrate became violently ill showing the characteristic symptoms from 30 to 90 minutes following ingestion.

Bacteriological examination of the milk from individual cows in the herd revealed the presence of the same hemolytic types of organisms in the udders of two of the animals. Periodic examination of the milk from these two animals revealed the interesting point that the numbers of staphylococci gradually decreased daily until none was present then suddenly large numbers appeared the following day. This was again followed by gradual diminution to zero when another showering of staphylococci occurred. The periodicity of these "showers" of staphylococci agreed in length with the periodicity observed in the epidemics among the students.

The importance of adequate refrigeration is emphasized by the fact that no cases of the disease occurred among the families occupying two residences on the campus where milk from the same herd was consumed. The milk delivered to these two homes was in small containers and efficiently refrigerated. The milk was delivered to the dormitories in 10-gallon cans and placed in a cooler operated at a temperature several degrees higher than prevailing in the electric refrigerators in the two homes. It is believed the more efficient cooling of the milk in these two homes retarded the development of the staphylococci sufficiently to prevent the disease among the consumers.

Bacteriological changes in acidophilus milk at room and ice-box temperatures. LENORE M. KOPELOFF, JOHN L. ETCHELLS AND NICHOLAS KOPELOFF, Psychiatric Institute and Hospital, New York. Jour. Bact. Vol. 28, No. 5, p. 489–500, Nov., 1934.

An important point in the sale and use of acidophilus milk is to retard the death of the cells after the inoculated milk has ripened. Early work

reported by these authors revealed that the acidophilus organisms diminished in numbers more rapidly at ice-box temperatures than when stored at room temperature. Since this is rather contrary to the usual practice of refrigeration in the preservation of food, the statement has been challenged and in some instances data submitted to show that the reverse is true. Acidophilus organisms may be divided into two physiological groups, described as "rough" (R) and "smooth" (S) according to the nature of the colony produced when the strain is grown on artificial media. The R type is known to be of therapeutic value, whereas there is considerable evidence to suggest doubt as to the therapeutic value of S type.

The authors subjected pure cultures of R and of S types to various storage temperature conditions and found that in general the R types (those of therapeutic value) died much more rapidly at ice-box temperature, whereas the S types tended to die less rapidly at the lower temperature.

Infectious bovine mastitis. 2. The streptococci of chronic bovine mastitis. Wayne N. Plastridge, E. O. Anderson, G. D. Brigham and E. H. Spaulding, Dept. of Animal Dis. and Dairy Husb., Conn. State College, Storrs, Conn. Conn. Agr. Exp. Sta. Bull. 195, March, 1934.

This study is based upon the examination of 208 strains of streptococci which were divided into 9 groups on the basis of methylene blue reduction, litmus milk reaction, ability to split aesculin, the sodium-hippurate test, and fermentation reactions. It was concluded that infectious chronic bovine mastitis is most commonly caused by a fairly well-defined group of streptococci, which should be regarded as a distinct species bearing the name *S. mastitidis*.

Of the 208 strains 87 per cent were from animals (with one exception) that had shown definite evidence of mastitis. Eleven per cent of the strains comprised a group differing from the previous one in that they fermented mannitol, reduced methylene blue milk, usually, and had a relatively low degree of virulence, causing a very mild form of mastitis. Animals infected with organisms of the first group rarely recovered completely, while those infected with organisms of the second group commonly recovered within a period from several months to a year.

In formulating plans of controlling mastitis due to streptococci a distinction should be made between animals harboring the two types of organisms described.

Chronic mastitis. G. J. Hucker and P. Arne Hansen, New York State Agr. Exp. Station, Geneva, N. Y. N. Y. Agr. Exp. Sta. Cir. 147, Aug., 1934.

This circular explains the two common types of mastitis (acute and chronic) and describes methods of determining the presence of the disease.

A summary of the directions given for bringing mastitis under partial control follows:

1. Test each quarter in the herd at regular intervals by either the strip cup or brom thymol blue test.
2. Segregate all cows reacting to either of these tests, as well as all cows giving milk that shows evidence of being infected.
3. All replacements should be tested (brom thymol blue) before being added to the herd. If replacement is dry, the advice of a competent veterinarian should be secured.
4. See that all cows are properly stripped out after milking.
5. Watch carefully all cows with injured quarters.
6. Sanitary precautions should be taken in the general management of the herd.
7. If trouble is still experienced after using the above methods, get in touch with your milk inspection laboratory or a veterinarian for a more intensive study of the herd.

The distribution of Brucella abortus in the infected udder. REDVERS THOMPSON. Canad. Pub. Health J., 25: 229, May, 1934.

A study of the milk from each quarter of the udder of fifteen cows showed that Brucella organisms were present in the right hind quarter of all but two, indicating a possible higher susceptibility here than in other quarters. The organism was continuously excreted with the milk from infected quarters during the lactation period unless clinical symptoms of mastitis appeared, when the *Brucella abortus* organisms disappeared.

Undulant fever due to Brucella of the porcine type: Report of a milk-borne epidemic. C. P. BEATTIE AND RAYMOND M. RICE, Council Bluffs, Iowa. Jour. Amer. Med. Assoc. Vol. 102, No. 20, p. 1670–1674, 1934.

During the months of February, March and April 1933, 30 cases of undulant fever appeared in epidemic form in the city of Council Bluffs, Iowa. Epidemiological and bacteriological investigations revealed that 27 of these 30 patients obtained their milk from the same dairy. The dairy herd involved supplied 80 homes; in 18 of these, cases of undulant fever developed. *Brucella suis* was obtained in blood culture from 6 of 14 patients and from the milk of one of the 20 cows in the herd. The epidemic ceased 13 days after the stoppage of the sale of the milk from this dairy. The authors conclude that *Brucella suis* has greater virulence than *Brucella abortus*.

Bovine tuberculosis. EDITORIAL. Jour. Amer. Med. Assoc., Vol. 102, No. 1, p. 48–49, 1934.

The death rate from respiratory tuberculosis in the United States has decreased every year since 1918 from 128.6 to 56.6 per 100,000 population in 1933. The death rate from non-respiratory tuberculosis decreased each year from 22.5 in 1917 to 6.7 per 100,000 in 1933. It is significant to note that these death rate values either increased or failed to decrease during 18 year period 1900–1918 and that the decline in the curves is coincident with the institution of an intensive program of elimination of dairy animals which react to the tuberculin test. Although this is obviously not the only factor involved, it is of interest to note that only 1.9 per cent of the 13,500,000 animals tested in 1932 were reactors, whereas 4.9 per cent were found positive in 1918. During the period from 1918 to 1933 there have been 115,170,388 animals tested and 2,693,570 reactors found.

Laboratory methods for the detection of milk from cows infected with mastitis. W. V. HALVERSEN, V. A. CHERRINGTON AND H. C. HANSEN, Univ. of Idaho, Moscow, Idaho. Jour. Dairy Science, Vol. 17, No. 4, p. 281, April, 1934.

The results of laboratory tests on milk from individual quarters of normal cows and cows suffering with acute and subclinical mastitis are reported. The tests made included the bacterial count on plain and blood agar, the type of infection, leucocytes per cubic centimeter, catalase, pH, chlorides, and grams of curd tension for the Hill's curd test. The various clinical tests used to detect pus were also applied to milk from udders known to be infected. More tests were also made to correlate the wide variation in curd tension which exists between quarters of the same udder with the various clinical observations made.

In general, the conclusions of this paper are as follows: (1) Acute mastitis produces such marked changes in the milk that it is easily recognized as being abnormal. The bacterial content, the leucocyte content, catalase content, pH value, chloride content and curd tension are all markedly affected. (2) Subclinical mastitis is very common; the milk generally appears normal and the condition generally goes unrecognized. A leucocyte content greater than 100,000 per cubic centimeter and a catalase content which produces more than 2.5 cc. of gas in 6 hours, according to the method described by Orla-Jensen, gave what appeared to be the most reliable indices of udder infection. (3) The addition of blood serum to milk reduces the curd tension much greater than the same degree of dilution by water. (4) Chemical tests commonly employed for the detection of pus in urine yielded negative when applied to milk from udders harboring subclinical mastitis. (5) Fifty-four samples of retail milk were tested; thirty-three samples contained excessive catalase and thirty-four samples contained more than 100,000 leucocytes per cubic centimeter. (6) These data emphasize the desirability of determining the presence of udder infection simultaneously with the curd tension.

Undulant Fever. J. L. MILLER. Ann. Int. Med. 8: 570, Nov., 1934.

A review of the laboratory and clinical work done on undulant fever shows that so far "the best preventive measure is pasteurization of milk." Efforts to immunize cattle or eradicate contagious abortion entirely are slow processes, and so far have not been successful. Infection in man may result from the use of raw dairy products from infected herds or from contact with infected animals. No successful form of treatment has been discovered, beyond rest, fresh air, nourishing food and treatment of local conditions which may arise. It is emphasized that diagnosis should not be based exclusively on any one of the numerous tests which are now in use, but two or more should be employed, as none give positive reactions every time.

Determination of carotene as a means of estimating the vitamin A value of forage. H. R. GUILBERT. Coll. of Agr., Univ. of California, Davis, California.

Karrer and Schlientz (Helv. Chim. Acta. 17: 7, 1934) have shown that carotene preparation from grasses, spinach, or nettles, is almost pure B carotene. Furthermore, no pre-formed vitamin A has been found in any plant source. B carotene is equivalent in biological value to vitamin A. Hence, the colorimetric test for B carotene which the author presents should reflect fairly accurately the vitamin A content of forage.

In general, the color values upon the carotene and xanthophyll as well as the unsaponifiable fractions varied with variations in the carotene content of the forage. Though the variations were not in all cases proportional to that of the carotene content the trend was sufficiently close to show that no serious error would be involved in comparing the biological value of different feeds on the basis of carotene, though xanthophyll or other substances contributed to the vitamin A activity. The results upon the samples used show that a product field cured under favorable conditions may closely approach the dehydrated meal in its vitamin A content.

Factors influencing the utilization of calcium and phosphorus of cow's milk. J. H. HESS, H. G. PONCHER AND HELEN WOODWARD, College of Medicine, Univ. of Illinois, Chicago, Illinois. Amer. Jour. Dis. of Children, Vol. 48, No. 5, p. 1058–1071, Nov., 1934.

One of the major problems in the use of cow's milk for infant feeding has been the suitable adjustment of the physical character of the curd. The three main factors governing the character of the curd formed in the child's stomach are the casein, rennin, and the calcium ions. Regulation of curd character involves a readjustment of one or a combination of these three factors. Recent clinical experiences with the natural "soft-curd" milk from selected cows indicate that its variability makes it less desirable than milk carefully adjusted under controlled conditions.

A recent method of adjusting the physical character of the curd has been to increase the amount of soluble calcium of milk by acidification with 0.3 per cent citric acid, then removing this calcium by ultrafiltration through a zeolite (sodium-aluminum silicate). The resulting product does not form a curd with rennet and it was found that about 20 per cent of the calcium had been removed. The work reported in this paper was conducted primarily to determine if milk produced by this base-exchange method carried enough calcium to keep the child on a positive calcium and phosphorus balance. The results showed that 100 cc of such milk per kilogram of body weight will keep a normal growing infant in a positive calcium and phosphorus balance. Although there is a reduction in the amount of calcium and phosphorus in milk treated by base exchange, the results of metabolism study indicate that the utilization of these elements is more efficient. The possible mechanism by which more efficient utilization takes place is attributed to three factors; (a) the size of the curd, (b) the state of the calcium, and (c) the reaction of the ash.

Special milk in the solution of the iodine deficiency problem. WM. D. WESTON, Columbia, South Carolina. Archives of Pediatrics, Vol. 51, No. 11, p. 683–690, Nov., 1934.

Previous work by the author has shown that in certain sections of the country where the amount of iodine in the soil is low, there is a definite parallelism between the iodine content of the vegetables grown and the milk produced. In these sections there is a high incidence of goiter, especially among children. There is ample evidence to indicate that the time most suitable to correct the iodine deficiency and thereby prevent goiter is during the early stages of childhood while the food consists largely of milk. Since it is possible to increase the iodine content of milk by feeding this element in suitable form to the cow, a study was made to determine the possibility of producing milk higher in iodine content in these so-called goiterous-regions. Carefully regulated feeding practices were instituted in those regions. Samples of the milk were analyzed periodically over a 10 month period as a check against seasonal variation. The results showed considerable variation in the iodine content of milk produced in various sections and in various seasons of the year. The highest iodine content of milk analyzed from Abbeville, S. C., was 1170 parts per million, and from Greenville, S. C., 1870 parts per million. Samples from Bainbridge, N. Y., and from Columbus, Wisconsin, showed maximum iodine values of 392 and 395 parts per million respectively.

Cooperative experiments were conducted with pediatricians in various parts of the country in which children of all ages were fed at predetermined levels of iodine intake. The data are not presented, but the conclusions from these feeding trials were (1) that children receiving milk high in iodine

content grew at a more rapid rate and more consistently than those on a low level of iodine intake. (2) by use of milk high in iodine it was possible to maintain a high hemoglobin content in premature twins and triplets. (3) weight increased upon a more nearly normal scale in the children receiving milk high in iodine. (4) habitual vomiting was successfully corrected by the use of this milk in all but one case. (5) milk high in iodine gave definite improvement of cases of debility and slow development. (6) a few cases of beginning goiter were corrected by the use of this milk, and (7) in the few cases studied, positive balances of calcium-phosphorus, and magnesium were maintained.

A milk and banana diet for the treatment of obesity. GEORGE A. HARROP, Baltimore, Maryland. Jour. Amer. Med. Assoc., Vol. 102, No. 24, p. 2003–2005, 1934.

The strict diet consists of 6 large well-ripened bananas and 1000 cc (about 1 quart) of skimmed milk to be eaten as three or more meals per day spaced according to the personal food habits of the patient. The diet is followed for 10 to 14 days and usually produces weight losses of from 4 to 9 pounds in persons who are active and carry on their usual living routine. Reduction of the diet to 4 bananas daily is well tolerated by some individuals and produces more pronounced reduction in weight.

After 10–14 days alterations may be made by substituting one or 2 eggs, some green vegetables, a little butter, a small bit of lean meat for one or two bananas. The diet as outlined is advocated because it is simple, cheap, readily available, palatable and effective.

Nutrition and Child-bearing. EDWARD MELLANBY, Univ. of Sheffield, Sheffield, England. Certified Milk Vol. 9, No. 95, p. 6, March, 1934.

Malnutrition during pregnancy is not necessarily due to the lack of sufficient energy-bearing food, but more commonly to the lack of certain factors essential to normal metabolism of the food ingested. Although there are, no doubt, many more factors involved in nutrition than the present status of knowledge reveals, it is well established that calcium, phosphorous, iodine, iron, and vitamins A and D are essential to normal metabolism. Evidence is rapidly accumulating to suggest that the toxemias of pregnancy such as albuminuria, spasmodic nausea, etc., may be the result of malnutrition.

As a general guide, the daily diet during pregnancy should include one quart of milk, one or two substantial servings of green vegetables, one or two egg-yolks, fresh fruit, 2 teaspoonfuls of cod liver oil, sea food twice each week and calf's liver once each week.

Pasteurized versus raw milk. Foreign Correspondent of London. Jour. Amer. Med. Assoc., Vol. 102, No. 22, p. 1860, June 2, 1934.

The report of a committee from the Royal College of Physicians which was unanimously adopted included the following items. (1) A daily ration of milk is important for the growth and health of children. (2) The risk of tuberculosis and other diseases following the consumption of raw milk is considerable. (3) Such risk can be obviated by the use of milk submitted to low temperature pasteurization. (4) Such pasteurization does not materially interfere with the nutritive value of milk. The college while realizing that milk should be produced from cows free from infection and under conditions of cleanliness recommends, (a) that local health authorities should be given power to require that milk sold within their areas should be pasteurized under official control, (b) that steps should be taken to permit the pasteurization and sale, as such, of milk from tuberculin-tested herds, and (c) that in areas where adequate pasteurization is now impracticable milk should be boiled before use.

The Milk Marketing Board (appointed by the government to control the milk industry) has produced a pure milk scheme to become effective in October, 1934. It is proposed to prescribe a standard of purity for milk, and producers who conform to it will receive a bonus on every gallon of milk. Funds for this bonus will be provided by a small levy on all producers of milk. The new grade will be termed "accredited milk." The farm, the cow, and the milk will be subject to expert examination by local health authorities. The object is not to produce a high-quality superior grade of milk, but to raise the general standard of milk.

Prevention of rickets by milk fortified with vitamin D from cod liver oil. WILLIAM R. WILSON, Yale University, New Haven, Conn. Jour. Amer. Med. Assoc., Vol. 102, No. 22, p. 1824–1831, June 2, 1934.

Thirty-three infants, most of whom were under 8 weeks of age at the beginning of the experiment, two-thirds of whom were either negroes or Italians, were fed special milk fortified with 150 Steenbock units of vitamin D from cod liver oil for a period of from three to six weeks. X-ray pictures revealed that 14 of the 33 infants remained normal, 17 developed slight rickets and two a moderate degree of rickets. When the number of units of vitamin D taken daily per 100 grams of weekly gain in weight was correlated with the degree of rickets, it was found that the average number of units taken by the nonrachitic infants was somewhat greater than the average number taken by those who showed slight rickets, and considerably greater than the number taken daily by the two infants who developed moderate rickets.

Since the requisite amount of vitamin D depends upon the rate of growth, and since some infants grow more than others, it is clear that rapidly growing infants may not receive adequate vitamin D in amounts of milk less than one quart per day. The data presented show that certain fast growing in-

fants who used less milk than others per unit of growth were not protected by milk containing 150 Steenbock units per quart. Minimum vitamin D standards should necessarily be determined by further work.

The prevention of dental caries. Lancet, Feb. 3, 1934, page 245. Diet and the teeth, Lancet, Feb. 3, 1934, page 257. Diet in relation to dental structure and disease. Brit. Med. J., Feb. 10, 1934, page 252. Diet and the teeth. Editorial. Brit. Med. J., Feb. 10, 1934, page 246.

These four papers summarize and discuss the third and last report by Mrs. Mellanby on her outstanding investigation of the development and preservation of teeth.

Beginning some seventeen years ago with a study of the dental structure of dogs and other animals, the third and most recent report of a clinical study on the teeth of 400 children confirms previous conclusions reached by the author that diet is a more important factor in the formation of sound teeth than is oral hygiene. A close relationship between the structure of the tooth and the incidence of dental caries was observed.

Examination shows that the large majority of the population of England has faulty tooth construction and any effort to correct this condition must result in a rather complete change in dietary habits. Milk, eggs, cheese, animal and fish fats, and vegetables should be used liberally and very little, if any cereals. Cod liver oil or some other source of vitamins A and D should be given to all children.

Nutrition in normal and abnormal pregnancy. New developments in relation thereto. J. C. HIRST. Pa. Med. J., 37: 377, Feb., 1934.

In a short paper discussing nutritional requirements in pregnancy the author recommends the inclusion of a quart of milk daily in a diet made up of cereals, fruits, vegetables and meat. Very little carbohydrate is given. The maintenance of an adequate calcium level is important and the supplementary administration of vitamin D is recommended.

Specially produced milk in the solution of the goiter problem. WILLIAM WESTON. South Med. Jour., XXVII: 249, March, 1934.

Adequate control of the goiter problems in children will come, according to this author, only through foods in which the organic iodine content is high. Of all foods, milk is the most universal and may carry a higher iodine content than others. Clinical studies in several sections of the country known for their high goiter incidence showed that when children were given a dried milk rich in natural iodine, symptoms of deficiency were almost entirely absent. Calcium and phosphorus values are favorably influenced by a high iodine intake, as are also growth and development. Irradiation has been found to increase the utilization of iodine, iron, calcium and phos-

phorus in milk and is, therefore, of value in the solution of the goiter problem. The product used in this study was Dryco.

Relation of diet to dental caries. H. F. HAWKINS. Jour. Am. Dent. Assn. April, 1934, p. 630.

The development of dental caries as a result of acids formed by fermenting starches may be prevented by maintaining the proper acid-base balance and calcium-phosphorus ratio. Adequate intake of certain elements as well as proper assimilation must be assured in order to keep a sufficient alkaline reaction in the body. Milk is important in increasing alkalinity and also provides sufficient calcium to fulfill the requirements. Furthermore, it increases assimilation and elimination of phosphorus. Fruits, vegetables, meat, fish and eggs also have important places in the maintenance of an alkaline reaction.

Vitamin D milk and the teeth. JAMES A. TOBEY. Dental Survey, March, 1934, p. 57.

The importance of calcium, phosphorus, and vitamin D in the formation of healthy teeth makes the increasing availability of vitamin D milk of outstanding importance to dentists as well as to physicians. The administration of a quart of vitamin D milk daily to all pregnant and nursing mothers, and to infants and children will not only prevent rickets but will insure better teeth in the children and will also help preserve the teeth of mothers.

Methods by which the vitamin D content of milk may be inereased are outlined, and the advantages of the product over cod liver oil, viosterol or other concentrates are pointed out. Experiments have shown that the potency of vitamin D milks per rat unit is much more efficacious than that of the concentrates. The danger from overdosage of vitamin D milk is so slight as to present no problem.

Dental caries III. Rickets in relation to caries in the deciduous and in the permanent teeth. A. F. HESS, H. ABRAMSON AND J. M. LEWIS. Amer. J. Dis. Child., 47: 477, March, 1934.

Observations on a group of 1167 children between the ages of 6 and 9 years, brought out the fact that the occurrence of rickets in infancy and of dental caries in the permanent teeth were not correlated. These children had been closely observed from infancy. Some had been protected from rickets by means of cod liver oil or other anti-rachitic, others had not. The deciduous teeth in the rachitic group showed a definitely higher incidence of caries than did the non-rachitic. Children admitted to the institution before one year of age showed fewer caries than did those admitted between three and four years. An interesting observation was made on a group of Negro children in New York City, among whom the incidence of rickets is much

higher than in the white races. These Negro children had permanent teeth much freer from caries than did white children.

The authors conclude that dental caries is not due to rickets but to nutritional disturbance in childhood and early adult life, as calcification of the permanent teeth does not begin until after the age when rickets commonly occurs.

Vitamin D, either as cod liver oil, viosterol or vitamin D milk, is essential for proper calcification of teeth and bones. It is suggested that "Calcifying" is a better descriptive term than "antirachitic" for this vitamin, the value of which goes far beyond the prevention and cure of rickets alone.

Vitamin D certified milk. W. E. Brown. Jour. of Med., XV: 88, April, 1934.

Bio-assays on certified milk produced on farms under its jurisdiction by the Medical Milk Commission of the Academy of Medicine of Cincinnati showed the milks to contain at least 160 Steenbock rat units per quart, which is better than that of 3 teaspoonsful of cod liver oil or 10 drops of viosterol daily. The commission states that physicians are safe in prescribing this milk for antirachitic purposes.

Relation of ingestion of milk to calcium metabolism in children. A. L. Daniels, M. K. Hutton, E. Knott, G. Everson and O. Wright, Iowa State College, Ames, Iowa. Am. J. Dis. Child., 47: 499, March, 1934.

A study of calcium, phosphorus and nitrogen retention in a group of children, 3 to 5 years of age, whose diets differed principally in the amount of calcium furnished by either 1 pint or 1 quart of milk daily, suggests that the physiologic differences in individuals may account for the fact that some do better on less milk than others do on more.

Children receiving plenty of fresh vegetables, eggs, fruits and meat as well as milk, and supplemented with viosterol or cod liver oil, may not need as much milk for optimum growth and development as do other children whose dietaries are not so well balanced or do not furnish sufficient protein, phosphorus and vitamins. It is further suggested that children whose dietaries are consistently on a high nutritional level do not show as great retention as do those whose bodies have been depleted through faulty diets over a long period of time.

Influence of fluorine ingestion upon the nutritional qualities of milk. P. H. Phillips, E. B. Hart and G. Bohstedt, College of Agriculture, Madison, Wis. Jour. Biol. Chem., 105: 123, April, 1934.

Although it has been shown that the ingestion of small amounts of fluorine is harmful to the teeth, the authors have been unable to demonstrate this fact in a series of experiments in which rats were given exclusive milk

diets from cows whose diets were supplemented with fluorine. No noticeable difference in residual fluorine of the body ash was observed in rats receiving milk from cows to whose diets fluorine had been added and rats receiving milk from cows whose diets did not contain added fluorine. It was further observed that the fluorine content of milk was difficult to influence by dietary methods.

A study of the relative antirachitic value of cod liver oil, viosterol and irradiated milk. T. G. H. DRAKE, F. F. TISDALL AND A. BROWN. Canad. Med. Assn. Jour., 31: 368, Oct., 1934.

A group of 529 infants in the city of Toronto were given antirachitics in the form of viosterol, cod liver oil, or irradiated vitamin D milk for a period covering five winter months. Presence of rickets was determined by means of x-ray. The infants were divided into two age groups, as younger, more rapidly growing babies are more susceptible to rickets, the younger group included all infants under four months of age, when the study was begun; the other group, infants from four to eight months. No moderate or severe rickets developed in any infants receiving from 1.5 to 12 drops of 250D viosterol, or irradiated milk containing 35 Steenbock units of vitamin D per 20 ounces, while only three out of the 137 receiving cod liver oil developed rickets. Difficulty in the administration of a suitable dose of cod liver oil may account for these three cases. One teaspoon of cod liver oil was as efficacious as three in preventing rickets. In conclusion the authors state that "in irradiated vitamin D milk we have a valuable addition to our present antirachitic armementarium."

A new concentrate for producing vitamin D milk with added vitamin A potency. FRANK J. HOLT, Health Products Corp., Chicago, Illinois. Milk Plant Monthly Vol. 23, No. 9, 1934.

Vitamin D milk is the most discussed food product on the market at present.

The Health Products Corporation of Newark, New Jersey, have developed a method of preparing a vitamin A and D concentrate by removing them in the non-saponifiable fraction which is about 1 per cent of the total fats. One pound of the concentrate will fortify (150 Steenbock units) 10,080 quarts of milk. Some secret steps in the manufacture are not presented.

The concentrate is emulsified into a small volume of milk which is added to the large volume of milk.

Vitamin D milk by the addition of refined cod liver oil concentrate. BION R. EAST, National Oil Prod. Company, Harrison, N. J. Milk Plant Monthly Vol. 23, No. 4, 1934.

The concentrate is prepared according to methods developed by Theodore

F. Zucker and patented for Columbia University. It has 1000 times the vitamin D potency of the original oil. It does not alter milk flavor.

The concentrate contains 900,000 Steenbock units per pound. One pound of the concentrate per 6000 quarts of milk is required to obtain the 150 Steenbock units desired in each quart of the milk. The concentrate is mixed with a small amount of milk. This is added to the main bulk of milk just before pasteurization.

Clinical tests have revealed that 50 Steenbock units of vitamin D per quart of milk are required to prevent rickets. Vitamin D milk prepared with the Zucker technique has been approved by the American Medical Association.

The concentrate is prepared by treating cod liver oil with 95 per cent ethyl alcohol, which dissolves the vitamin D and some of the fatty acids. From the alcoholic solution the fatty acids are saponified and precipitated as calcium soaps. This mass is treated with acetone. The acetone solution containing the vitamin D is separated, concentrated and treated with ether. The ether is finally distilled off leaving a waxy, tasteless and odorless product containing a high concentration of vitamin D.

A comparison of the nutritional and growth values of certain infant foods. C. T. WILLIAMS AND A. O. KASLER. Jour. Ped., 4: 454, April, 1934.

Sixty infants divided into three groups of 20, each group given a different formula, were compared with each other and with a control group of 20 breast fed infants as to nutritional condition, growth, incidence of respiratory and other infections, and presence of rickets. The three formulas used were (1) certified milk, milk sugar, lime water and water; (2) evaporated milk, milk sugar and water; and (3) Mariott and Schoenthal's lactic acid evaporated milk and corn syrup formula. The study was undertaken to determine the comparative advantages of fresh and canned milks for infant feeding.

The infants were observed for 6 months and during that time received cod liver oil and orange juice routinely. A study of the nutrition and growth of these 60 infants indicates that fresh and evaporated milks are equally successful in infant feeding, but that the superior digestibility of evaporated milk due to the heat treatment caused fewer digestive upsets in the groups receiving this form of milk. For infants under 3 months it was found necessary to dilute one-third the Marriott lactic acid formula to prevent digestive disturbances.

A vitamin B deficient ration. R. C. BENDER, G. E. FLANIGAN AND G. C. SUPPLEE, The Dry Milk Company, Bainbridge, N. Y. Jour. Nutrition Vol. 8, p. 357, 1934.

Autoclaved yeast or yeast concentrates are most commonly employed as a source of vitamin G in vitamin B deficient rations. Milk is relatively high in vitamin G and low in vitamin B. The authors find that dry whey autoclaved for 2 hours at 120°. (248° F.) contains no vitamin B, as indicated by the growth of white rats, and that 15 per cent of this dry whey in feeds supplies an amount of vitamin G sufficient for growth. In their work they obtained more consistent and satisfactory results with this product and a source of vitamin G free from B than from autoclaved yeast.

The vitamin B supplementation of milk. G. C. SUPPLEE, R. C. BENDER AND G. E. FLANIGAN, The Dry Milk Company, Bainbridge, N. Y. Jour. Nutrition Vol. 8, p. 365, 1934.

That vitamin B affects growth and efficiency of food utilization seems to be an observed fact. These authors found that there is a direct linear relationship between the rate of growth of white rats and the amount of vitamin B supplied in the ration.

Dry milk containing a concentrated water extract of vitamin B obtained from polished rice showed greater growth promoting properties than was shown by the milk alone. Vitamin B with or without milk supplementation increases the efficiency of food utilization as judged by growth response per unit of food intake. Potent vitamin B extracts may be prepared from rice polish by simple extraction procedures, and these may be condensed in vacuum, spray dried or dried by the Just process without significant diminution in potency.

The fat-soluble vitamins and dental caries in children. C. D. MARSHALL DAY AND H. J. SEDGWICK, Univ. of Rochester, Rochester, N. Y. Jour. Nutrition Vol. 8, p. 309, 1934.

The diets of 147 of 318 children of approximately 13 years of age were supplemented with 6000 U.S.P. units of vitamin A and 1470 Steenbock units of vitamin D in the form of viosterol. The remainder of the group (171) were kept under observation as checks. No attempt was made to in any way interfere with the food intake. From the results the conclusion is drawn that without altering the diet the administration of vitamins A and D to children of this age and within the period of investigation (15 mos.) has no appreciable effect upon the rate of progress of the caries process in teeth already erupted or which erupt during the experimental stage.

Vitamin Studies. XIX. The assimilation of carotene and vitamin A in the presence of mineral oil. R. ADAMS DUTCHER, PHILIP L. HARRIS, EWA R HARTZLER AND N. B. GUERRANT, Penn. State College, State College, Pa. Jour. Nutrition 8, p. 269, 1934.

Former work by Dutcher, Ely and Honeywell have shown that rats are unable to utilize vitamin A in butterfat when the latter is dissolved in min-

eral oil. Later work showed that this generalization was not applicable to vitamin A in cod liver oil which was utilized more efficiently than that from butterfat under similar conditions.

In the present work these conclusions were confirmed, especially at the lower levels of vitamin A feeding. The harmful effect of mineral oil can be explained on the basis of carotene excretion from the body in the unabsorbed mineral oil. This hypothesis is supported by the fact that the yellow pigment excretion is roughly proportional to the carotene ingested. This is not true when carotene is fed in the absence of mineral oil.

Irradiated milk: Characteristics of the flowing film required for optimum efficiency of antirachitic activation. G. C. SUPPLE, The Dry Milk Company, Bainbridge, N. Y., and M. J. DORCAS, The National Carbon Co., Inc., Cleveland, Ohio. Jour. Dairy Science Vol. 17, No. 8, p. 527, Aug., 1934.

The flow of milk films over smooth vertical surfaces by gravity can be differentiated as films with dominant smooth flow characteristics, or dominant turbulent flow characteristics.

Milk may be activated to a 2+ degree of calcification, as determined by standard assay procedures under a wide range of properly coordinated conditions involving film capacity, film thickness and distance of film travel, within a momentary period of exposure. There are certain optimum conditions wherein the applied energy is utilized more effectively and efficiently; such conditions are determined by the relationship between film thickness and film capacity.

Results indicate that the irreducible minimum in time necessary to give the specified degree of activation desired in these experiments was from 0.75 to 1.30 seconds. Exposure periods of 2.70 seconds gave the same degree of activation under conditions which permitted a marked increase in the amount of milk which could be activated per unit of time.

The relative efficiencies of irradiated ergosterol and irradiated yeast for the production of vitamin D milk. W. E. KRAUSS, R. M. BETHKE AND WILLARD WILDER, Depts. of Dairy and Animal Ind., Ohio Agr. Exp. Sta., Wooster, Ohio. Jour. Dairy Science Vol. 17, No. 10, p. 685, Oct., 1934.

Two groups of Holstein cows were fed 60,000 rat units of vitamin D as irradiated ergosterol and 60,000 rat units of vitamin D as irradiated yeast daily. Two other groups of Holstein cows were fed 120,000 rat units of vitamin D from the same sources daily.

Vitamin D assays of the milk produced by these groups of cows showed that the irradiated ergosterol was approximately two-thirds as efficient in allowing transfer of its vitamin D to the milk as was the irradiated yeast.

No satisfactory explanation for this was found. It could not be attributed to a difference in absorption from the intestinal tract since the vitamin D content of the feces and of the bloods was the same regardless of the supplement fed. The existence of different forms of vitamin D having different species effects is considered as a possible explanation, as is also a difference in the "disappearance" into the tissues of vitamin D from the two sources.

Irradiated milk: The reflecting properties and antirachitic activation as affected by the impingment angle of the incident radiation. G. C. SUPPLE, The Dry Milk Company, Bainbridge, N. Y., and M. J. DORCAS, The National Carbon Company, Cleveland, Ohio. Jour. of Dairy Science Vol. 17, No. 9, p. 607, Sept., 1934.

This investigation was planned to show the degree to which the activating rays from ultra-violet light are reflected from milk surfaces as this knowledge is important in an understanding of the radiant energy needed to activate milk.

The reflection of ultra-violet light from milk surfaces was of the same order as reflections from water. The extent of reflection increases with increased angle of impingment. At 45 or 77° C. the reflection is constant being 6 per cent for a 30° angle; 13.4 per cent for a 20° angle, 28 per cent for a 15° angle, 34 per cent for a 10° angle and only 3 per cent when the rays struck the milk film at right angles.

Vitmain D studies. E. C. MCBEATH. Amer. J. Pub. Health, 24: 1028, Oct., 1934.

A group of more than four hundred children in institutions were given various forms of vitamin D milk in an effort to determine the influence of this vitamin on the incidence of dental caries. The milks used included fresh and evaporated, with the vitamin D contents increased by means of a cod liver oil concentrate added direct to the milks. Three different levels of vitamin D were given: 100, 150 and 300 Steenbock units daily. The author concludes that vitamin D is important in the nutritional control of dental caries and that there is a probable correlation between the amount of vitamin D given and the degree of control of dental caries.

The production of vitamin D milk by the feeding of irradiated yeast. C. A. SMITH, Director Dry Yeast Dept., Standard Brands Inc., New York City. Milk Plant Monthly, Vol. 23, No. 5, 1934.

Dr. Smith points out that three methods of increasing the vitamin D content of milk are available. During 1930–31 more than 50 per cent of the white infants and over 75 per cent of the negro infants attending the New York City Health Stations showed definite signs of rickets. Dr. Mellanby in England has shown that an abundance of vitamin D prevents or delays tooth decay. Rickets cause defective bone and teeth structures.

Deformed pelves of girl babies as a result of rickets cause untold misery and perhaps death in bearing their children in later life.

Yeast is a food. It contains 50 per cent protein and is rich in ergosterol. Irradiation of yeast changes the ergosterol to vitamin D so that one pound contains 450,000 Steenbock vitamin D units. This yeast has 70 times the vitamin D potency of cod liver oil. The accepted standard for cod liver oil is 13¼ Steenbock units per gram (453 grams equal 1 pound).

Feeding yeast to cows improves their general appearance and health. It also stimulates their appetites. Observations of this nature have been made over a long period of time in 25 herds with a total of more than 1,000 cows.

The irradiated yeast is mixed with the grain ration. Low producing cows require less yeast than high producers although high producing cows convert more efficiently the vitamin D of the yeast into the milk. Age, breed, period of lactation and like factors do not influence Vitamin D potency of the milk. Irradiated yeast must be fed to cows for 15 to 20 days before maximum vitamin D potency of the milk is obtained. After 10 days 80 per cent of the maximum is obtained.

A license must be obtained from the Wisconsin Alumni Research Foundation, Madison, Wisconsin, before a dairyman is allowed to feed irradiated yeast. This requires that the dairyman have a high local rating, and when the license is obtained the Fleischmann Laboratories of Standard Brands, Inc., 595 Madison Avenue, New York City supply and cooperate in the successful use of the treated yeast.

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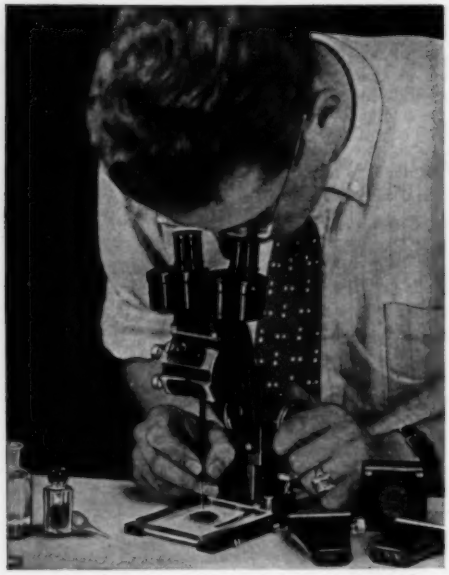

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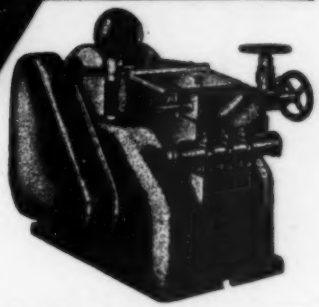

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Specialized Wyandotte Products have been developed to meet all conditions of equipment, water, and other factors which enter into cleaning. Discuss your cleaning problems with your Wyandotte Service Representative. He is always glad to work with you to secure maximum efficiency in all your cleaning operations.

Your jobber can always supply you with Wyandotte. Remember, Wyandotte is your assurance of efficient cleaning.

The J. B. Ford Company **Wyandotte, Michigan**

Bacto-Nutrient Agar

Bacto-Nutrient Agar is recommended for the routine plate counts of bacteria in milk. When it is prepared for use in the laboratory it conforms in every respect to the standard medium recommended for this purpose in "Standard Methods of Milk Analysis."

Results obtained with plates of medium prepared from Bacto-Nutrient Agar are reliable and comparable.

Bacto-Malt Agar

Bacto-Malt Agar is prepared expressly for use in determining the mould and yeast counts of butter and other dairy products, and for determining the sanitary conditions of manufacture.

When made up for use in the laboratory it has a reaction of pH 5.6, and may easily be adjusted to a more acid reaction if desired.

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